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THE RÔLE OF THE CELLS OF SCHWANN IN THE FORMATION OF TUMORS OF THE PERIPHERAL NERVES *

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The origin of tumors of the peripheral nerves has long been a matter of controversy. Two schools of thought are still opposed, the one deriving these tumors from the connective tissue, the other from the cells of Schwann. The former opinion goes back to von Recklinghausen,⁵⁹ the latter is usually derived from Verocay,⁵⁸ although he was not the first to propose a schwannian origin for these tumors. At the present time the connective tissue hypothesis is defended by Penfield³⁷ and the schwannian theory by Masson.²⁹ This controversy had never seriously engaged our interest until recently the hazard of the clinic placed in our hands complete autopsy material from 2 remarkable cases of multiple tumors of the nervous system which seemed to promise opportunities for forming an opinion based on personal investigation. Our observations on this material form the primary basis of this study.

METHODS

The subjects of this study were two unrelated girls, 15 and 16 years of age respectively at the time of death. Complete autopsies were performed in each instance; 4½ hours after death in 1 case (B. S.), and 12 hours postmortem in the other (E. N.). There were also surgical specimens from each case fixed immediately at the moment of removal at operation. In the 1st case (B. S.), numerous fragments of various nerves and nerve roots were fixed

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in formalin, formol-Zenker, Orth's, Bouin's, and Zenker's fluids, absolute alcohol, ammoniated alcohol, and Domenici's and Laguesse's (formula J) fixatives and Weigert's Gliabeize. The material being ample, transverse and longitudinal sections from each nerve were made and stained by the following methods: Cajal's reduced silver, Nageotte's hematoxylin, Herxheimer's scarlet red, Freeman's silver method for nerve fibers, Mallory's phosphotungstic acid hematoxylin, Loyez's method for myelin, Weil's method for myelin, Laidlaw's and Perdrau's methods for connective tissue, van Gieson's, Foot's silver method for connective tissue on Zenker-fixed material, the method of Gros-Bielschowsky, cresyl violet, neutral ethyl violet-orange G, hematoxylin and eosin, mucicarmine, Bodian's method for nerve fibers, Ranson's method for unmyelinated nerve fibers, Regaud's and Cowdry's methods for mitochondria, the methods of Jakob, Doinikow, and many others in certain instances. The brain and spinal cord were fixed by immersion in formalin after removal of blocks from the hypothalamic tumor and from the spinal cord for fixation in Zenker's fluid, alcohol and formalin bromide. In addition, preparations from the skin were made with Bloch's dopa reaction. The brain and spinal cord were later studied after staining with the usual methods used by neuropathologists following fixation in formalin (10 per cent).

In the 2nd case, certain peripheral nerves, notably the ulnar and median nerves in the arm, the lower cord of the brachial plexus, the upper cord of the lumbar plexus, the vagus and sympathetic trunks in the lower thorax and the ilioinguinal nerve, were removed and fixed in the same manner for comparison with those of the 1st case, although they were not grossly abnormal. The brain and spinal cord were fixed by immersion in 10 per cent formalin together with the associated tumors of meninges and nerve roots. Tumors from the upper cervical region and intracranial meninges, however, had already been removed at operation and studied after immediate fixation in 10 per cent formalin, Zenker's fluid and the formalin bromide solution of Cajal. In addition, abundant material from other cases of all sorts of tumors of the central and peripheral nervous systems, collected over many years in the pathological laboratories, were used for comparative study. We also repeated the classical experiments of Nageotte and produced tumors in rabbits by autotransplantation of peripheral nerves.

We finally made numerous preparations from normal nerves by all these methods for comparison.

We must confess at the outset that we did not obtain profitable results with any of the methods of Nageotte on our pathological material. Perhaps the material was unsuitable, perhaps our lack of familiarity with the methods was at fault. The best preparations for axis cylinders were obtained with the methods of Bodian⁷ and Freeman.¹⁹ The former method gave consistently usable preparations; the latter was more fickle, impregnating often neurokeratin, but when it did give a specific impregnation the preparations were perfect for our purposes since the connective tissue was completely colorless and could be counterstained by the method of van Gieson. Sharply specific preparations of neurokeratin we obtained with Mallory's phosphotungstic acid hematoxylin on formalin-fixed paraffin sections by omitting to treat the sections with oxalic acid and permanganate. In these preparations any fragment of a myelin sheath stood out sharply blue against a uniform salmon pink background. They were found very difficult to photograph, but perfect for study. Many excellent methods for connective tissue were available, both for staining (Masson, Mallory) and for impregnation (Perdrau, Laidlaw, Foot). The method of Foot, when used on Zenker-fixed material, impregnated much more than the methods of Laidlaw and Perdrau when used on formalin-fixed material from the same region. The sections prepared by Masson's trichrome method on Bouin-fixed material gave beautiful preparations which we found, however, very difficult to photograph, so that for this purpose we were obliged often to have recourse to the ancient but excellent method of van Gieson. Often we wished we might have colored drawings made, but the expense was too great and besides we wished, if possible, to record our observations by actual photographs rather than by drawings, necessarily to a variable extent interpretative. If the number of photographs be found excessive, we can only plead our belief that one good photograph is more useful than many pages of verbal description.*

Finally, we regret the lack of any utilizable specific method for

* We must here record our indebtedness to our photographer, Mr. Francis T. Harmon, without whose skill with filters many of our observations could not have been adequately recorded by photographic means.

staining or impregnating the cells of Schwann. After repeated but fruitless efforts with the methods devised by Cajal, Nageotte, Dockrill and others, we abandoned them in despair. A specific method for these cells would immediately resolve many uncertainties and obviate much indirect argumentation, however plausible. We will proceed, therefore, immediately with the description of our observations, made with the admittedly imperfect methods at our disposal, and afterwards try to determine how far any valid conclusions may be drawn from them concerning the rôle of the cells of Schwann in the formation of tumors of the peripheral nerves.

CASE REPORTS

CASE 1. *Clinical History:* B. S., a girl aged 12 years, was referred to the clinic on Feb. 5, 1929 (Unit No. 9648), complaining of failing vision. (Previously briefly reported from a clinical standpoint by Bailey, Intracranial Tumors, Case 36.)

In 1927 it was noted at school that she could not read from the blackboard. She had complained of backache a year previously and had worn a sacro-iliac belt for 6 months, but had otherwise been well except for repeated attacks of asthma. The family history was unimportant except that the father had over his body numerous manifestations of generalized neurofibromatosis. The mother, one brother and one sister were free from such malformations.

The patient was slender but healthy in appearance. On the skin were numerous brownish patches varying in size from 1 to 3 cm. in diameter. There were also numerous, small, violet colored soft elevations about 1 cm. in diameter. Small, firm subcutaneous nodules could be felt along the course of the right median, right supraorbital and left greater auricular nerves. Over the anterior aspect of the right ankle was a boggy mass 5 cm. in diameter in which firm discrete nodules could be felt. The optic nerve heads were normal but vision was much reduced. No other abnormality of the nervous system was found. A diagnosis of generalized neurofibromatosis complicated by glioma of the optic chiasm was made. An X-ray of the head was made in an attempt to see the optic canals but it was not successful.

The child was taken to another clinic where, on March 4, 1929, a right frontotemporal exposure was made, disclosing a tumor involving the chiasm and both optic nerves. The nodule in the right cubital fossa was also exposed and was found to involve the median nerve so firmly that it could not be removed.

She returned on July 5, 1929. The vision at this time was found to be $R = 0.1$ and $L = 0.6$. There were bizarre temporal defects in each visual field. The fundi appeared normal.

Between July 9, 1929, and Jan. 29, 1930, she was given roentgen radiation directed toward the optic chiasm through the temporal regions. The visual fields did not change, the acuity very little. On Oct. 18, 1930, the right acuity was 0.4-2 and left 0.6-2. On Jan. 6, 1930, an X-ray of the optic canals demonstrated enlargement of both of them, the right being slightly larger than the left. On April 3, 1931, the basal metabolism was found to be -11

per cent. She weighed at that time 63.4 kg. and was 162 cm. in height. On Nov. 27, 1932, she had grown slightly stouter. The optic discs were slightly pale, but seemed otherwise unchanged. On May 29, 1933, she came complaining of attacks of pain behind and under the left ear. Her speech was a little thick and there was some atrophy of the right half of the tongue. She then weighed 66.2 kg. and was 162 cm. high. She had been menstruating regularly for 2 years. Vision was unchanged. On June 3, 1933, audiometer tests demonstrated normal hearing in both ears, and caloric tests showed both labyrinths to be functioning normally.

She was admitted to the hospital on Oct. 31, 1933 because of increasing difficulty in swallowing. At this time she weighed only 56.2 kg. and her height was 164 cm. The basal metabolic rate was -3 per cent. The cutaneous manifestations previously noted had not changed much except that numerous peripheral nerves could be palpated under the skin. The breasts were small. The bodily hair was normally abundant. The menses were not disturbed. There had never been any polyuria. The visual acuity and visual fields were as previously noted in October 1930, but the optic discs looked paler. The pupils were round, equal, 5 mm. in diameter and reacted well to light. There was a nystagmus on looking to right and left, slower and coarser to the left. Convergence was fair. Hearing was normal in both ears to audiometer, and normal caloric responses were obtained in each ear. External ocular movements were normal. Motor and sensory fifth nerves were intact bilaterally. There was possibly slight weakness of the seventh nerve on the right side. Sensation over the right side of the pharynx was diminished. The right palatal and pharyngeal muscles were weakened and the right vocal cord paretic. The sternomastoid and trapezius muscles seemed of normal strength bilaterally. The right half of the tongue was atrophied. Speech was slurred and the patient was much troubled by accumulation of mucus in the throat which she could swallow only with difficulty. The muscles of the right arm and leg seemed slightly weaker and at times a dorsal plantar response was obtained on the right side. No defect of general sensation was demonstrated over the body. The gait was slightly unsteady and she fell constantly to the left and backward in Romberg's position. There was no definite incoordination of the extremities.

A diagnosis of neurofibroma of the right twelfth nerve, compressing the ninth and tenth nerves and the bulb, was made. On Nov. 4, 1933, an attempt was made to expose the posterior fossa, but it had to be abandoned because of inability to flex the head forward so as to expose the region. An attempt to anesthetize with ether in the hope that relaxation of the muscles would enable the head to be flexed forward resulted in cessation of respiration. The patient afterward complained of pain in the back and right gluteal region. It was planned to make a roentgenogram of the spine, but she continued to have difficulty with breathing and suddenly at 5:00 A.M. on Nov. 7, 1933 she died.

Postmortem Examination

Autopsy was performed 4½ hours postmortem. Acute distention of the heart, a menstruating uterus, and bilateral follicular cysts of the ovaries were the insignificant findings in the viscera,

except for the alterations of the nerves. The vagus nerves were enlarged from the foramina jugularia to their finest ramifications. In fact, every nerve in the body, so far as could be seen, was enlarged in irregular fashion, often looking like a string of beads. The vagus nerves at the level of the bifurcation of the trachea measured approximately 1 cm. in diameter. The phrenic nerves also varied from 0.5 to 1 cm. in diameter. The sympathetic chains were in places as much as 3 cm. in diameter. It is futile to describe all the nerves; down to their finest ramifications they were clearly visible, appearing as though injected and distended by some milky fluid. The nerves of the extremities were generally enlarged and contained fusiform swellings which reached as much as 3 cm. in diameter.

When the brain was removed the convexity appeared practically normal; perhaps the convolutions were slightly flattened. There were no tumors of the meninges. The olfactory bulbs were normal. Both optic nerves and the optic chiasm were greatly enlarged. In the angle between the bulb, pons and cerebellum on the right side was a firm nodular tumor 3 cm. in diameter. It appeared to arise from the twelfth nerve and there was an extension of the tumor through the canalis hypoglossi which was transected when the brain was removed. The tumor indented the bulb deeply and to a less extent the cerebellum and pons. The third, fourth, fifth and sixth cranial nerves were normal. The seventh, eighth, ninth, tenth, eleventh and twelfth cranial nerves on the left side were normal near their origin, but the ninth, tenth, eleventh and twelfth nerves swelled suddenly just before passing through their foramina of exit. On the right side the seventh and eighth nerves were elongated because of distortion by the tumor of the twelfth nerve, but were otherwise normal. The ninth, tenth and eleventh nerves swelled suddenly at their foramina of exit, as did those on the left side. The right twelfth nerve could not be identified, but the tumor before mentioned projected through the canalis hypoglossi.

On median sagittal section of the brain the tumor of the optic chiasm was seen to obliterate the infundibulum, extending from the mammillary bodies to the anterior commissure. On cross section of the cerebrum the tissue appeared normal. No tumors, macrogyria or other abnormalities were found. In the cerebellum, how-

ever, there was found in the left hemisphere, just back of the dentate nucleus, a grayish tumor 1.5 cm. in diameter.

All of the roots of the spinal nerves were more or less swollen, some of them bearing intraspinal tumors of considerable size. The third thoracic nerve on the left side bore a tumor 1.5 cm. in diameter. The cauda equina looked like a cluster of grapes, all of the roots bearing nodules of tumor in strings. The filum terminale was slightly enlarged, but had no gross tumorous nodules. These tumors were clearly arising from the roots of the nerves and not from the meninges.

Microscopic Examination

The soft subcutaneous mass above the right ankle proved on microscopic examination to be a lipoma. It was not otherwise remarkable except that the nerve trunks in the connective tissue septums were grossly enlarged. The nerve fibers were few and either widely separated from one another or collected into a small portion of the cross section by a great overgrowth of the supporting tissue. This overgrowth consisted mainly of a great number of spindle cells with scanty cytoplasm and elongated dense nuclei. The cells were accompanied by myriads of reticulin fibrils. In other nerves the cells of the overgrowth were thicker, with sometimes abundant cytoplasm and larger oval nuclei. This supporting tissue had in places undergone a degenerative change resulting in a finely granular precipitate between the cells.

The pigmented areas of the skin were not remarkable except that there was an almost unbroken row of cells in the stratum germinativum clearly demonstrated by Bloch's dopa reaction.

The purple, soft subcutaneous lesions had undergone extensive degeneration. Between the cells was permeated everywhere a homogeneous glassy material which stained faintly blue with hematoxylin and also with aniline blue. In this homogeneous jelly-like material floated various cells, strands of collagen and blood vessels. In areas that had not undergone this degeneration could be seen cells with considerable cytoplasm, some of them very elongated, others round, others club shaped, lying in irregular masses and separated by a variable amount of reticulin or collagen. These cells resembled very much the cells in nevi described by Masson. The homogeneous material seemed to arise by degeneration of these cells.

The optic nerves consisted mainly of a dense gliosis divided into funiculi by septums of connective tissue extending inward from the sheath. In the outer funiculi were a few myelinated nerve fibers, particularly in the inner margin of the left nerve and the upper and inner margins of the right nerve. Comparison with Freeman preparations was convincing that many more nerve fibers persisted than the myelin sheath preparations would lead one to suppose, a few fibers being found even in the innermost funiculi. The glial scar was composed of piloid astrocytes usually elongated in the long axis of the nerve, although there was a condensation of neuroglial fibrils often along the septums of connective tissue which ran at various angles to the main direction of the fibers of the gliosis.

The optic chiasm continued without interruption into the tumor of which it formed the anterior margin. The tumor was composed principally of large bipolar spongioblasts. It was surrounded laterally by a dense gliosis containing concentric masses of calcification, and passed over anteriorly by gradual transition into the gliosed optic nerves. In the anterior margin of the tumor there were many nerve fibers both myelinated and unmyelinated. There was no fatty degeneration either in the optic nerves, chiasm or tumor. The gliosis around the tumor contained great numbers of the so-called Rosenthal fibers. Within the brain was found, in addition to the spongioblastoma of the optic chiasm and hypothalamus, a round subcortical tumor in the cerebellum, 1.5 cm. in diameter, having the typical structure of a protoplasmic astrocytoma. Also, in the molecular layer of the cerebellum were nodules of abnormal cells seeming, from their reactions to impregnation methods, to be composed of both neuroglial and microglial cells. These nodules had no relation to blood vessels and were numerous in the molecular layer of the cerebellum. No such abnormal accumulations could be found in the cerebral cortex, but often, particularly in the frontal lobes, were seen abnormal accumulations of oligodendroglia and neuroglia along the walls of small blood vessels. In the spinal cord, no pathological alterations of the interstitial cells were found, but often there was a considerable collection of pathological cells at the point of exit of the anterior spinal roots which extended as much as 2 mm. within the cord. These cellular clumps formed numerous fibrils of reticulin

and appeared like small neurinomas. The cerebral cyto-architectonics seemed to us to be normal and the nerve cells and nerve fibers unaltered. The intracranial leptomeninges and blood vessels were microscopically normal.

The tumor arising from the second cervical nerve root was 2 cm. in diameter. There was very little fatty degeneration in the tumor, only a few fat-containing macrophages being seen along the walls of the vessels. Throughout the tumor were nerve fibers, usually in groups but often widely scattered. Some of these had no myelin sheaths, but most were myelinated. These fibers were not accumulated at the periphery of the tumor. There was a thin capsule of the tumor composed of parallel strands of collagenic connective tissue. The cells of the neoplasm had very little cytoplasm. Many of them were elongated, with their long axes parallel to the nerve fibers. Whenever nerve fibers were cut longitudinally in the sections the tumor cells were also cut longitudinally and *vice versa*. The tumor cells were accompanied by vast numbers of delicate collagenic fibrils. Their nuclei were the typical, crenated, wrinkled fibroblastic nuclei so often drawn by Maximow. Where the nerve fibers were cut transversely it could be seen that these collagenic bands collected around the nerve fibers, but many such collections contained no nerve fibers. The tissue was very loose, being dissociated apparently by edema. There were great numbers of loose cells of rounded or slightly elongated form which were free of any association with the nerve fibers or collagenic bands. Many of these looked like lymphocytes but most had larger nuclei resembling those of ordinary connective tissue cells. Many were undoubtedly macrophages and had fine granules of fat in their cytoplasm. These loose cells ran the entire gamut of Maximow's polyblastic series. Very few cells could be seen that had any resemblance to Schwann cells. There were a few ganglion cells present. Some were surrounded by capsular cells, but others by what appeared to be cells very similar to the other tumor cells forming abundant collagen. A few fenestrated cytoplasmic bands were found containing unmyelinated nerve fibers, undoubtedly Remak fibers. Rarely a collagenic bundle would contain a fat nucleated cell without myelin sheath or nerve fiber, possibly a Buengner cord. There was no tendency whatever for the cells to form whorls about the nerve fibers. Blood vessels were scanty, composed of endothelial cylinders surrounded

by a few strands of collagen. Most of the intercellular substances reacted to stains and impregnations similar to collagen. There was very little reticulin and no elastin.

Numerous spinal roots had similar tumors of greater or lesser size. Their structure was so similar that repeated descriptions are unnecessary. Within them were found occasionally formations resembling Wagner-Meissner corpuscles.

The larger tumor on the twelfth cranial nerve was composed of spindle shaped cells with a fair amount of cytoplasm. The nuclei varied from oval forms resembling closely those of ordinary fibroblasts to long sausage shaped forms with denser chromatinic accumulation. These nuclei showed very little tendency to lie in palisades. No mitoses were found. The cells lay in broad interlacing bands. Myriads of reticulin fibrils were laid down between the neoplastic cells running always in the direction of the long axis of the cells. No fatty degeneration was found anywhere in the tumor. No persisting nerve fibers were found.

The left vagus nerve within the cranial cavity measured 4 mm. in diameter. It was dissected down through the jugular foramen and a segment about 1.4 cm. in length was removed. The lower end contained a group of ganglion cells of the ganglion jugulare. These ganglion cells appeared fairly normal. Two types could be distinguished, one group being smaller and darker than the others. The cells contained granular tigroid material and some lipochrome pigment. They were surrounded by capsular cells. The nerve itself was thickened by a great overgrowth of collagenic tissue for the most part. The myelinated nerve fibers were disseminated throughout the cross section; sometimes lying nearer together, at others widely separated. Very few cytoplasmic bands were seen in the center of collagenic accumulations. There seemed to have been very little destruction of the nerve fibers. Around many of the fibers the collagenic bundles were increased. A few areas filled with a loose connective tissue composed of fibroblasts and cells of the polyblastic series were present. These areas seemed very edematous, the cells being widely separated, the polyblasts lying in a loose meshwork of fibroblasts. In other areas the tissue was very dense, thick bands of collagenic fibrils with fibroblastic nuclei dispersed among them lying parallel to the nerve fibers.

The right vagus nerve in its intracranial extent being essen-

tially similar in structure to the left vagus nerve no separate description is necessary.

Along the spinal nerve roots and along the roots of the cauda equina were nodules of various sizes which had the typical structure of neurinomas. They were composed of tenuous spindle cells, running in streams, which formed myriads of delicate reticulin fibrils. Usually these nodules were too large to permit of any opinion concerning their origin, but some were found of small size and these (Fig. 1) could be seen clearly to arise often eccentrically in the roots. One was found (Fig. 2) which unmistakably was developing in the arachnoid. After examining great numbers of sections of the nerve roots, there was no doubt left in our minds that these small neurinomatous nodules arose almost invariably in relation to the perineurium. A few small nodules within the nerve roots had no obvious relation to the perineurium.

The microscopic alterations in the peripheral nerves may be well illustrated by a description of the upper cord of the lumbar plexus. In preparations for nerve fibers and for myelin sheaths, it could be seen that the nerve fibers were dispersed in irregular fashion throughout the cross section of the funiculi, either singly or in groups. At times most of the nerve fibers would be collected into the center of the funiculus, at others largely around the periphery, but usually in irregular fashion throughout. Whenever the nerve enlarged markedly, the nerve fibers obviously diminished in proportion, so that the enlargement of the trunk was not due to increase in number of nerve fibers. Nor were ganglion cells present, so that the enlargements were not due to the formation of ganglions such as cause enlargements of the normal sympathetic chain.

Many of the nerve fibers were normal, others in all stages of degeneration. All of the alterations so well described by Cajal, Nageotte, Jakob and others could be identified in the myelinated nerve fibers; it is unnecessary here to describe them in detail. We will remark only the presence of end stages. Throughout these nerves were found cytoplasmic nucleated cords of the size of a large myelinated nerve fiber. The cytoplasm was stained yellow by van Gieson's and red by Masson's trichrome method. Each such cytoplasmic cord was surrounded by a sheath reacting similar to collagen. These cords were usually isolated but sometimes in groups (Figs. 3, 4, 7, 16). We believe them to be Buengner cords

resulting from hypertrophy of the Schwann cells after the degeneration of myelinated nerve fibers, so well described by Nageotte and recently by Masson. Hereafter such formations, for the sake of brevity, will be referred to by this name without further description. The axis cylinders themselves were found, in these degenerated areas, swollen and fragmented in typical fashion. The unmyelinated nerve fibers were found in clusters, lying in the fenestrated cytoplasm of Remak cells (Figs. 11 and 17). These unmyelinated fibers we found always running parallel (Fig. 10), and in dozens of sections we could never find any anastomoses or divisions of these fibers. Swellings from time to time occurred along their course (Fig. 24), but never did they seem to pass outside the Remak bundles. In degenerated areas the Remak bundles were often greatly hypertrophied, their cytoplasm thickened and the unmyelinated nerve fibers absent (Figs. 13 and 14). Often they were transformed into more or less homogeneous masses which stained like collagen but more faintly (Figs. 21 and 22). Around each myelinated nerve fiber and around each Remak band were numerous fibrils of reticulin or collagen which were impregnated by Perdrau's method (Fig. 20) and stained red by van Gieson's method. These fibrils could be seen clearly in close association with the sheaths of Schwann around the myelin rings and seemed to us to be adherent to the outer surface of the Schwann sheaths rather than within them. These fibrils form the well known Plenck-Laidlaw sheath and were often greatly increased in numbers which, remaining after the disappearance of the nerve fibers, appeared as thick cords of collagenic fibrils. Such accumulations could also be seen about the Buengner cords. When Foot's method was used on Zenker-fixed tissue, the cytoplasm of the Remak bands was impregnated *in toto* (Fig. 19) and in degenerated areas where the Remak bands were hypertrophied they were also stained blue by Masson's trichrome and red by van Gieson's method. It is possible that many of these large fenestrated structures that contained no unmyelinated nerve fibers may have been hypertrophied Buengner cords secondarily subdivided by collagenic partitions as described by Masson so well in amputation neuromas (Figs. 5 and 6).

Between the nerve fibers and associated structures (Buengner cords, Remak bands, Schwann sheaths) in addition to the fibrils

of the Plenk-Laidlaw sheaths there was a vast accumulation of cells and material which seemed to us to be clearly connective tissue in nature. Typical fibroblasts with the wrinkled crenelated nuclei so often drawn by Maximow were omnipresent (Figs. 23 and 26). Often large areas of dense, pure fibroblastic proliferation could be found (Fig. 26). Cells of the polyblastic series were scattered about and bundles of collagenic fibrils twisted and writhed in every direction among the other elements which were often separated widely by the accumulation of a homogeneous or finely granular material staining very feebly by any method and giving only a faint reaction to mucicarmine (Fig. 16). This myxoid edematous material seemed to be formed by degeneration of the interfibrillary connective tissue; the process could be followed in all stages. Nothing resembling a neurinomatous formation was found. Sometimes a condensation of cells occurred at the periphery of a funiculus; usually no distinction could be seen between the connective tissue in the periphery and center of a funiculus so that no definite perineurium could be said to be present. The cells in the periphery had the same fusiform shape as the interfibrillary ones and sometimes seemed to form a syncytium.

The left median nerve measured 1.4 by 1 cm. in diameter in the upper arm. The only fat found in the cross section of the nerve was contained in large fat cells in the epineurium. The epineurium appeared normal, but the rest of the nerve was decidedly abnormal. Many of the funiculi were enormously enlarged. Many funiculi had an easily recognizable perineurium consisting of densely packed collagenic bundles in which were interspersed elongated nuclei, but in most of the funiculi the tissue of the inner surface of the perineurium became looser and proliferated, its interstices filled with a coagulum which often took a bluish tinge in preparations stained with hematoxylin and eosin. This proliferation sometimes occupied as much as two-thirds of the cross section. Usually the separation between the perineurium and the nervous fasciculi was sharp, but in places, and in certain funiculi, there was no distinction between perineurium and endoneurium. In some funiculi this loose connective tissue with the accompanying bluish coagulum permeated throughout the cross section, in others only one-half or a smaller portion of the funiculus was involved. At one point on the inner surface of the perineurium of a large funiculus was a

proliferation of cells that bore a very close resemblance to a neurinoma. Any part of the cross section of a funiculus might be the seat of a proliferation of the endoneurial connective tissue with a great proliferation of reticulin and collagen, the whole grossly swollen apparently by the accumulation of a colloidal material in the interstices. Myelinated nerve fibers were scattered throughout, but there were areas, where the connective tissue proliferation was most intense, in which no nerve fibers were to be found and even in areas where the nerve fibers were most numerous there were septums of connective tissue containing no nerve fibers. Around each myelin sheath was a ring of material giving the reactions of collagen, of greater or less thickness. Usually distinct collagenic fibrils could be made out running in the same direction as the nerve fibers, but often the sheath appeared homogeneous, even when impregnated with silver. Often a large mass of cytoplasm would be found to contain one or more small myelinated fibers and several unmyelinated fibers. Such a mass looked fenestrated on cross section when stained with Foot's method, phosphotungstic acid hematoxylin or the trichrome stain. Many of the fenestrations contained no nerve fibers. Nothing resembling Buengner cords of degenerated myelinated fibers could be identified. The great increase in size of the nerve seemed to us to be due clearly to proliferation of the endoneurial and perineurial connective tissue plus a tremendous accumulation of edematous fluid which did not give any reaction in this case for mucin.

In the cubital fossa the median nerve suddenly enlarged to form a swelling 1.8 cm. in diameter in fusiform fashion about 2 cm. in length. In this region the nerve fibers were present only in the extreme periphery of the cross section. The rest of the tumor was made up essentially of a loose connective tissue. The spindle shaped cells associated with bands of collagenic fibrils were widely dissociated by an edematous fluid represented in the sections by a delicate coagulum which in some areas became a homogeneous glossy hyalin. This material did not give the reaction of mucin to mucicarmine or only very faintly so. In the interstices of the collagenic meshwork were loose cells running the entire gamut of the polyblastic series. The nuclei of these cells were typical connective tissue nuclei, wrinkled and crenelated. Here and there one saw homogeneous columns with smaller nuclei which we have

interpreted as sclerosed Remak columns. A few Buengner cords in the periphery of the tumor near the nerve fibers were seen.

The right ulnar nerve in the cubital fossa measured only 7 mm. in diameter. There was no fat except in the epineurium. The structure was in no wise different from that of the median nerve so that a separate description is unnecessary.

The right sympathetic chain was greatly thickened, measuring as much as 5 mm. in diameter even between ganglia; no fat was present except in the epineurium. The ganglion cells with their capsular cells were clearly recognizable (Fig. 25), also the bands of unmyelinated fibers with their accompanying Remak cells. Occasionally a myelinated fiber was found. But these structures made up only a small part of the cross section. The greater part of the increase was due to a proliferation of fibroblasts. In some areas there was no admixture of nervous elements, only fibroblasts and cells of the polyblastic series being present, together with bands of collagen and a great amount of granular and glossy débris which stained faintly for mucin. In some areas the fibroblasts formed very little collagen, in others dense masses of fibrils. Many of the Remak bundles had lost their nerve fibers, especially those widely scattered in the fibroblastic areas, and others had undergone a sclerotic change and been transformed into homogeneous masses which stained rather faintly like collagen.

The right ilioinguinal nerve varied in diameter from 3 to 5 mm. In the thickest parts the cross section was made up almost entirely of fibroblastic proliferation which was continuous with the perineurium without any recognizable transition. The connective tissue was very edematous for the most part, interspersed with hyalin and granular coagulum, and masses of collagenic fibrils. Around the periphery of the cross section a few myelinated nerve fibers and many Remak bundles persisted, some of which were sclerosed and had lost their axis cylinders. The myelinated fibers were often surrounded by dense masses of collagenic fibers running parallel with the nerve fibers.

The greater auricular nerve showed changes similar to those found in the ilioinguinal nerve.

CASE 2. Clinical History: E. N., a girl of 13 years, was admitted to the University of Chicago Clinics on May 2, 1934 (Unit No. 103364) complaining of paralysis of the arms and legs.

Some 18 months previous to admission she began to complain of weakness of the right arm and leg. For nearly a year she had had difficulty in starting urine. She complained somewhat of pain in the back of the neck. She never complained of headache. In February, 1934, she began to drag the right foot. She was taken to a hospital where a lumbar puncture was made. After this she grew rapidly worse. The right arm and leg became paralyzed and the left arm and leg progressively weaker. An X-ray of the head demonstrated an irregular patchy erosion of the cranium in the left parietal region, which looked like a syphilitic lesion. In spite of negative Wassermann tests on the blood and cerebrospinal fluid, antiluetic treatment was begun, but the patient grew steadily worse and began to have difficulty in breathing. A biopsy of the lesion in the skull showed it to be due to erosion by a meningeal tumor.

On admission she was a tall, well developed girl, 5 feet, 8 inches high, weighing 152 pounds, with large pendulous breasts. She was unable to sit up, stand or walk. Vision was normal in both eyes. The optic discs were normal and visual fields full. The right pupil was slightly larger and reacted sluggishly to light. The left pupil reacted promptly by direct and consensual stimulation. There was a very fine and rapid nystagmus in each eye on looking to right and left. The external ocular movements were normal. There was a right facial weakness on voluntary movement, of peripheral type. Both sternomastoid muscles were weak; the right more so. The right trapezius muscle was paralyzed; the left was very weak. The tongue was normal; also the palatal and pharyngeal musculature.

The patient could scarcely raise her head from the pillow. No voluntary movement could be made with right arm or leg; the left arm and leg were very weak. All extremities were flaccid but the tendon reflexes were all present except the right ankle jerk. The right knee jerk even seemed exaggerated. There was also a bilateral dorsal plantar response. The abdominal reflexes were present on the left side and absent on the right. There was a sustained patellar clonus on both sides, but a clonus was obtained only at the left ankle. The sensory findings varied greatly at different examinations; most constantly found were astereognosis and inability to recognize letters and numbers in the right palm, and diminution of vibratory sensitivity over the right half of the body. At times a sensory loss to pinprick could be found on the left side with a level about C 3 to C 5.

With the laryngoscope both vocal cords could be seen to move freely. Under the fluoroscope both diaphragms could be seen to move paradoxically. X-ray of the skull demonstrated again the irregular erosion of the left parietal bone, yet the findings indicated that the neurological symptoms were predominantly due to some lesion in the spinal canal above the origin of the phrenic nerves. Lipiodol was injected into the lumbar sac. Queckenstedt's test was positive, the fluid was clear, and two lymphocytes were present. Under the fluoroscope, as the patient was tilted head downward, the oil stopped temporarily at L 1, then moved rapidly upward to C 5. Lipiodol injected into the posterior cistern remained there and would not descend into the cervical canal.

On May 10, 1934, under local anesthesia with novocaine, the posterior fossa of the skull was opened and a laminectomy made of C 1, 2, 3 and 4. This exposure was very difficult because the head could not be flexed forward. When the posterior cistern was opened it could be seen that there was a tumor anterior to the spinal cord extending from the foramen magnum to the lamina of the third cervical vertebra. The spinal cord was stretched like a tent over

its posterior surface. The tumor was exposed by sectioning the first and second cervical nerves on the right side and rotating the cord to the left. The tumor was attached firmly to a broad base anteriorly. It was removed piecemeal and the bed curetted with a sharp curette. The wound was then closed and the head, neck and shoulders immobilized by a plaster cast. There had been no respiratory or other difficulty during the procedure. Microscopically the tumor proved to be a meningioma.

The patient improved gradually until May 15th. The paresis of the left arm and leg was not increased but more definite sensory loss could now be demonstrated below C 4. On May 15th she began to have a septic temperature. Leukocytosis was 16,500. The wound was clean, however, and the fever soon subsided. By May 24th she could flex the right elbow and the diaphragm was moving well on each side. On June 1st she was discharged. At this time she had recovered the ability to move the right arm and leg. The dorsal plantar response was present on the right side but not on the left.

On Oct. 28, 1934, she was readmitted to the hospital. She was then able to walk long distances. The right pupil did not react to strong light directly or consensually. It was slightly larger than the left pupil which reacted both directly and consensually to light. The left pupil reacted to accommodation; the right did not. There was a rapid nystagmus of both eyes on looking to the left and upward; none on looking to the right. The external ocular movements were normal. There was no facial weakness. The sternomastoid and trapezius muscles contracted strongly on each side. All tendon reflexes were present except at the right ankle, somewhat brisker on the right side. There was a dorsal plantar response on the right side only. There was no clonus at ankle or patella. No sensory loss of any kind was detected over the body or extremities. The grasp of the right hand was slightly weaker than that of the left, and its thenar and hypothenar eminences slightly flattened, otherwise the muscles of the extremities seemed symmetrically and normally strong. The patient walked and ran normally but the right leg tired more quickly. Visual acuity of the right eye was 0.8-3 and of the left eye 0.2-1. The right optic disc was slightly redder than normal, flat, with normal cupping and well defined borders; the left optic disc was also slightly redder than normal but the upper and nasal borders were slightly elevated. An X-ray of the skull did not reveal any extension of the parietal lesion. The optic foramina seemed of normal size.

The patient was discharged and returned Jan. 28, 1935, at which time her condition was practically the same. She weighed now 77.5 kg. The Babinski sign on the right side had disappeared. On Feb. 19, 1935, under avertin anesthesia supplemented with ether, the eroded area in the left parietal bone was encircled and the bone infiltrated by tumor was removed with a rongeur. The dura mater was then opened laterally and reflected to the longitudinal sinus. There was only a thin sheet of tumor a few millimeters in thickness inside the dura mater. The dura mater was removed up to the lateral wall of the sinus. Some tumor on the lateral wall was cauterized. In doing so the wall was penetrated. Smart bleeding was stopped with muscle. The scalp was closed over the defect, the dura mater being left open. The tumor was proved by microscopic examination to be a meningioma thoroughly infiltrating the dura mater.

There was subsequently slight weakness of the right hand, and numbness and tingling of the right hand and right side of the lips which increased until

February 22nd. There was also difficulty of expression which began to improve about February 25th and rapidly disappeared. The wound healed well except for one persistent sinus which had not entirely closed when she was discharged on March 27, 1935.

She remained perfectly well until June 28th when she had a generalized convulsion. On July 24th she had a series of 8 convulsions. She was examined on Aug. 27, 1935, at which time the ocular findings were unchanged and the right arm and leg seemed weaker but there was no Babinski sign. She was given phenobarbital, 30 mg. thrice daily. On Aug. 25, 1935, there was a definite right hemiparesis with positive Babinski sign. There was also a definite forced grasping in the right hand. There had been no more convulsions.

She was readmitted to the hospital on Oct. 10, 1935, complaining that she could not think well. The left parietal area was bulging but soft. The left optic disc was elevated about 1 D. The ocular findings were otherwise unchanged. There was a right hemiparesis with positive Babinski sign and forced grasping of the right hand. No sensory loss could be detected over the body. On Oct. 17, 1935, a ventriculogram was made by inserting a needle through the defect in the left parietal region. This demonstrated an evagination of the left lateral ventricle toward the parietal defect, the ventricular wall extending to within 1 cm. of the surface of the brain. The right lateral, third and fourth ventricles were also markedly dilated. No air was in the posterior cistern but some globules of lipiodol still remained in this region. It seemed that there must be an obstruction in the posterior fossa of the skull, so on Oct. 21, 1935, a suboccipital operation was undertaken under avertin anesthesia supplemented with ether. The left lateral ventricle was punctured early and fluid in abundance escaped. The operation progressed uneventfully until the cerebellar hemispheres were exposed. Suddenly the cerebellum began to herniate through the wound. Puncture of the cerebellar hemispheres revealed no hematoma; the needle in the lateral ventricle was draining well. It was impossible to explore around the sides of the cerebellum. No explanation of the sudden pressure could be found. It was impossible to proceed with the exploration. The wound was closed over the tense cerebellum. The patient naturally was much distressed, developed respiratory difficulties and died on Oct. 28, 1935.

Postmortem Examination

Autopsy was performed 12 hours postmortem. Apart from the nervous system there was found an acute purulent bronchitis and early bronchopneumonia, acute distention of the right side of the heart, endometrial polyp and a menstruating uterus. The dura mater was adherent to the brain in the left parietal region. The posterior surface of the cerebellum was badly lacerated. There was a marked internal hydrocephalus, especially of the left lateral ventricle, as noted in the ventriculogram. A small nodule of tumor 4 mm. in diameter was found on the inner surface of the dura mater just back of the right frontal sinus. A small warty tumor was present on each third nerve just at its entry into the dura

mater. A larger similar tumor was attached to the dura mater back of the posterior clinoid process where the left fifth nerve penetrated this membrane. A tumor 5 mm. in diameter lay in the left internal acoustic meatus and another 1 cm. in diameter in the right acoustic meatus. A similar tumor 5 mm. in diameter lay in each jugular foramen. The sixth, fourth and twelfth nerves were not accompanied by tumors. Two other nodules lay on the dura mater of the suboccipital fossa at some distance from the foramens. Several small nodules of tumor of varying sizes were found on the falx cerebri and tentorium cerebelli. Opposite the bodies of the first and second cervical vertebrae was a mass of tumor 3 by 5 by 2 cm. in diameter which had dislocated the spinal cord backward and to the left but had not compressed it. It was attached by a broad base to the dura mater anteriorly. At the level of the body of the sixth cervical vertebra lay another tumor 1 cm. in diameter on the posterolateral aspect of the spinal cord attached to, but not arising within, the spinal root. There were numerous nodules of tumor a few millimeters in diameter on the roots of the cauda equina. The leptomeninx was greatly thickened throughout the length of the spinal cord. Many of the dorsal roots were swollen slightly in fusiform fashion but no tumors of any size of the spinal roots were found. Many of the roots of the cauda equina looked like strings of small beads. One small nodule was found on a motor root in the upper lumbar region.

Microscopic Examination

The left oculomotor nerve was enlarged to 4 mm. in diameter. At its largest diameter a transverse section showed by microscopic examination that most of the nerve fibers were pushed to one side but others were scattered around most of the periphery. Occasional bundles and isolated fibers were seen deep within the cross section. The greater part of the cross section was occupied by a tumorous proliferation of spindle shaped elongated cells with scanty cytoplasm and elongated nuclei containing abundant chromatin. These cells formed broad bands interlacing in every direction. The nuclei were irregularly distributed with little or no tendency to lie in rows. In some areas the cells formed whorls. In many of the whorls one could identify an axis cylinder (Fig. 27) sometimes with a myelin sheath (Fig. 34). In most of the larger

whorls no nerve fibers could be identified, but often a large cell with clear cytoplasm and a vesicular nucleus looking like a Buengner cord could be seen (Fig. 33). In the non-tumorous areas the axis cylinders, myelin sheaths and endoneurium were easily recognized. The endoneurium was often thickened and hyalinized. But it was very difficult to recognize anything that might have been Schwann cells. Rarely one could find what appeared to be a sheath distinct from the endoneurium. In the whorls it was often possible to distinguish two portions — an outer, denser ring staining heavily with eosin, and an inner proliferation, semisyncytial in character and with more delicate protoplasm, staining less densely with eosin (Fig. 38). The outer dense ring was continuous with the endoneurium, the inner cells were of a more delicate cytoplasm. The cells of the tumor where they ran in bands resembled closely those of the endoneurium of the uninvolved portion of the nerve. There was no definite boundary between tumor and uninvolved nerve. The cells of the tumor formed abundant fibrils of reticulin. So did the cells in the outer layers of the whorls. The inner portions of the whorls were often entirely free from reticulin (Fig. 28).

The right third nerve measured 5 mm. in diameter at its widest point. It was more completely transformed into tumor but numerous nerve fibers persisted around the entire periphery. Otherwise conditions were very similar to those in the left third nerve and no special description seems necessary.

The grand sympathetic trunk appeared microscopically to be quite normal. The right ilioinguinal nerve appeared normal microscopically. The lower cord of the left brachial plexus was normal microscopically. The upper cord of the lumbosacral plexus was normal microscopically except for one funiculus which contained in its center a tumorous proliferation. The perineurium of this funiculus was greatly proliferated, there being a dense outer portion resembling a normal perineurium and then a proliferation of loose fibrous tissue five to ten times as great in diameter as the normal perineurium. Then followed a layer of apparently normal nerve fibers, within which was the tumorous proliferation that occupied more than one-half the cross section of the funiculus. The affected funiculus was enlarged to twice the diameter of the next largest funiculus in the nerve trunk. Within the tumor one saw

clearly two types of tissue — elongated cells in great whorls and bands, and a reticulated, apparently syncytial tissue. The latter tissue resembled closely the Remak bands of the uninvolved portion of the nerve; the former the endoneurial cells. Often within one of the large whorls would be found a small amount of the reticulated tissue enclosing a myelin sheath or naked nerve fiber. The tumorous mass was fairly sharply outlined but there was no capsule and many nerve fibers and myelin sheaths persisted within the growth.

There appeared to be no diminution of the myelinated nerve fibers in the optic nerves peripheral to the chiasm.

One of the flat nodules in the falx cerebri was sectioned. It proved to be a dense, disc-like mass of collagenic tissue, containing very few nuclei, lying between the two layers of the dura mater. Another larger one was a typical psammomatous meningioma.

Sections at various levels along the spinal cord demonstrated that the leptomeninges were greatly thickened along the entire spinal cord. Occasionally small tumors were found in the dorsal roots, rarely on the motor roots. These tumors looked sometimes like typical neurinomas, and sometimes were composed of whorls of cells similar to a meningioma. In addition rarely a root would be seen with a different appearance; in these roots the nerve fibers and myelin sheaths had almost entirely disappeared and their places were filled by a trabeculated, lightly stained cytoplasm (Fig. 32). A small amount of fibrous tissue subdivided this trabeculated material into cords. The appearance was very similar to that of the neuroma forming on a transected peripheral nerve.

The tumor of the right vagus nerve measured 1 cm. in diameter. It looked microscopically, for the most part, like a typical neurinoma. But in some areas small whorls were formed so that the tissue here had the appearance of a meningioma, (leptomeningial endothelioma, meningothelioma). In some of these whorls axis cylinders could be identified (Fig. 29). The nerve fibers lay around the periphery or penetrated in bands of considerable number into the growth. Myriads of reticulin fibrils permeated the tumor everywhere.

The tumor of the left trigeminal root measured 12 mm. in diameter. It had a sort of hilum into which the nerve plunged to

break up into bands of fibers which passed through the tumor in all directions. The tumor tissue had all the appearance of a typical neurinoma. The cells lay in broad bands and formed myriads of delicate reticulin fibrils.

The right acoustic tumor measured 16 mm. in diameter, the left 12 mm. The right tumor was a typical acoustic neurinoma for the most part but in small areas whorls were formed (Fig. 37). The left acoustic tumor was very similar (Figs. 35 and 36).

The tumor of the left vagus nerve in the intracranial cavity measured 6 mm. in diameter. It had evidently arisen within the nerve because no nerve trunk was found on the surface of the growth, but myelinated fibers were scattered in the peripheral layers of the tumor. The tumor was an inextricable mixture of the two types of tissue we have been describing, consisting of broad bands of elongated delicate cells with elongated nuclei tending to lie in rows, separating masses of other cells forming small whorls. The whorls were not cross sections of the elongated cellular bands. Such cross sections were also found and were readily distinguished from the whorls. Many of the whorls originally formed around nerve fibers, since in some instances such whorls with myelin sheaths in the center were found in the periphery of this tumor.

The spinal cord was surrounded by a thickened and densely collagenic pia-arachnoid membrane within which the nerve roots were often widely dispersed. Around these roots could be found arachnoidal clusters and within them neurinomatous nodules. The fourth lumbar dorsal nerve root on one side contained a typical neurinoma about 1 mm. in diameter (Fig. 39 (N)). The delicate, greatly elongated cells of this tumor ran in long bands; the nuclei showed a definite tendency to align themselves in rows and the cells were forming numerous reticulin fibrils. In the opposite posterior root was a similar neurinomatous formation of larger size which extended into the posterior horn of the spinal cord (Fig. 39). The reticulin fibrils of the tumor followed into the spinal cord almost to the central canal in great numbers. The intramedullary tumor reproduced the true neurinomatous tissue and was not what is ordinarily called a "neurinoma centrale" in which no reticulin is produced.

In addition there was a small tumor of entirely different appearance in one funiculus. This small tumor was composed exclusively

of small tight whorls of cells, giving it the appearance of a meningothelioma (Fig. 40). It was, however, entirely within the nerve root and, although it reached the perineurium, did not appear to have arisen from this structure which was clearly distinct. Although the tumor was fairly sharply circumscribed, it had no definite capsule and within it were nerve fibers scattered about both within and without the whorls.

Moreover, the posterior raphe of the spinal cord was open in places almost down to the central canal. The tumor at the first cervical level, which had been partially removed at operation, contained considerable scar tissue and hemorrhage. It was composed of strands of dense cytoplasm seeming to anastomose like those of cardiac muscle, the strands being separated after the manner of an accordion. In this meshwork of cytoplasmic strands the numerous nuclei were embedded with no tendency to palisading. Blood vessels were rare. The neoplastic cells did not form reticulin or collagen. The spinal cord had been pushed to one side, compressed and gliosed at this level.

The tumor at the sixth cervical level was firmly attached to the dura mater over a broad base. It resembled in many parts the tumor at the first cervical region, but in addition it contained numerous whorls of cells often hyalinized and occasionally calcified (Fig. 30). In some areas these whorls were so numerous and compact as to resemble closely the similar formations in the nerve roots.

The meningeal tumor over the frontal dura mater resembled so closely in certain areas an acoustic neurinoma that it could with difficulty be distinguished.

The external surface of the brain did not appear abnormal except over the parietal herniation. Otherwise the convolutions appeared normal. Nor did the ventricular surfaces appear abnormal except underneath the parietal hernia. There were no nodules or abnormal irregularities of the ventricular surfaces. On cross section nothing abnormal could be seen with the naked eye, but microscopic studies revealed very extensive abnormalities of the finer structure of the cerebral hemispheres and basal ganglia. Nothing abnormal was found in the cerebellum or bulb, but scattered throughout the cerebral cortex and subcortical regions were collections of abnormal cells a few millimeters in diameter. In the

subcortex they appeared clearly to be large distorted neuroglial cells with vascular processes extending to vessel walls. But in the cortex the nature of these cells was not so clear. They had nuclei which were most often vesicular, like those of neurons, but varied greatly in size. The cells gave off numerous processes similar to neuroglial cells and these processes were not impregnated by Bielschowsky's method nor by the gold sublimate method of Cajal, at least in Globus' modification. They were impregnated by the silver carbonate method of Hortéga if the impregnation were pushed beyond the limit of specificity. The cells contained no Nissl bodies. These cells seemed clearly to be of the same nature as the abnormal cells so often described in tuberous sclerosis. In addition the cyto-architectonics of the cortex were grossly deranged in the neighborhood of these abnormal accumulations of cells. Displaced and abnormally oriented nerve cells were often found in the deeper layers of the cortex or even in the subcortex.

The nodules on the roots of the cauda equina were microscopically typical neurinomas with nerve fibers penetrating for a short distance only in the peripheral portions. Although rather sharply circumscribed in some areas, the tumor cells penetrated diffusely between the nerve fibers for 1 or 2 mm. beyond the apparent margin.

DISCUSSION

Such cases of multiple tumors of the nature of those we have just described are not excessively rare and have been recorded by Agostini,¹ Schnyder,⁴⁵ Bielschowsky and Rose,⁶ Penfield and Young,³⁸ Orzechowski and Nowicki,³⁶ van Bogaert,⁵⁷ Foerster and Gagel,¹⁶ Struwe and Steuer,⁵⁴ Schairer⁴³ and many others. The association of these various pathological alterations is therefore due to more than chance. There must be some underlying cause for their frequent association in the same patient. This cause has been sought in a common embryological origin for the affected structures. In order to evaluate this hypothesis, it is necessary to make a detour into the structure and development of the nervous system.

Anatomy: It is not needful here to enter into controversial matters or to review the history of the development of our present views concerning the structure of nerves. These matters can be

found discussed in detail in the masterly studies of Nageotte.³⁴ Suffice it to say that we must recognize in the nerve trunks three categories of structures: (1) the nerve cells, axis cylinders and myelin sheaths; (2) the capsular cells of the ganglia, the Schwann cells, and Remak cells; and (3) the endoneurial-perineurial connective tissue. The endoneurium and perineurium have essentially identical cellular composition, being composed largely of fibroblasts which form interstitial fibrils of reticulin, collagen, and rarely of elastin, together with a variable number of other cells of connective tissue origin. Those collagenic fibrils of the endoneurium which run longitudinally in the intervals between the nerve fibers are known as the fibers of Key-Retzius. The entire endoneurial-perineurial system constitutes the sheath of Henle if we read that ancient author correctly. But more important for our present study is another structure which we will follow Nageotte in calling the Plenk-Laidlaw sheath, since these authors (Laidlaw²⁶) have so clearly demonstrated it. This structure is a delicate network of argyrophilic fibrils which closely invests the medullated nerve fibers and may even extend with them for a short distance into the spinal cord. We will return to this sheath later. The myelin sheath is doubtless a part of the axis cylinder, since it is formed also around such nerve fibers in the central nervous system, but Speidel⁴⁸ has recently demonstrated clearly that its formation is precipitated by the proximity of cells of the second category in the peripheral nerves. These latter cells migrate out along the developing nerve fibers and apply themselves to the surfaces to form sheaths about the fibers which have long been known as the Schwann cells of the myelinated fibers and the Remak cells of the unmyelinated fibers. These cells are believed by Nageotte to form a syncytium. If so, Speidel's studies indicate that the syncytium is a secondary formation. It matters little for our present purposes. More important is the fact that on the outer surface of the Schwann cells is a condensation called the neurilemmal sheath or membrane of Schwann.⁴⁶ Such a condensation about the Remak cells is less evident. It should be remarked here that, although the neurilemmal sheath is easily seen in adult normal nerves, it is extraordinarily difficult to demonstrate clearly the cytoplasm of the Schwann and Remak cells. Moreover, there is some doubt concerning the relation of the membrane of Schwann

to the Plenk-Laidlaw sheath. Masson²⁹ is inclined for various reasons given in his papers to believe that the Plenk-Laidlaw sheath is an argyrophilic network within the membrane of Schwann and coterminous with it. This would make it a sort of basement membrane around the Schwann cell, and quite a nuisance for the present study since it is best demonstrated by methods that also demonstrate sharply the interstitial substances formed by the fibroblasts of the endoneurium. It is quite obvious, in the absence of any utilizable method of differentially emphasizing the cytoplasm of the Schwann and Remak cells, that the problem of differentiating the origin of tumor cells from Schwann cells or endoneurial cells should be particularly aggravated by the fact that they both form interstitial substances reacting in identical fashion to staining methods. This fact is essentially the crux of the controversy between the two schools of thought represented at the present time by Penfield³⁷ and Masson.²⁹

Embryology: We have above remarked the tendency to explain the association of multiple disseminated tumors involving the nervous system by assuming that the structures in which the neoplastic transformation occurs have a common embryological source. It may be well at this point to enumerate the most important types of pathological alterations concerned. They include such apparently disparate lesions as tuberous sclerosis of the brain, glioma of the optic chiasm, melanoblastosis of the leptomeningx, leptomeningotheliomas, neurinomas of the acoustic nerve, neurinomas of the peripheral nerves and their roots, interstitial hypertrophic neuritis, disseminated neurofibromatosis, cutaneous nevi, cutaneous hyperpigmentation, subcutaneous elephantiasis, and many others. All of these pathological formations have been related to normal structures associated with the nervous system by various authors, the hyperpigmentation to the cells of Langerhans (Masson³²), the nevi to Wagner-Meissner corpuscles (Masson³⁰), the disseminated neurofibromatosis to the endoperineurium (Penfield and Young³⁸), the interstitial hypertrophic neuritis to the Schwann cells (Boveri⁸), the neurinomas to the Schwann cells (Verocay⁵⁸), the leptomeningotheliomas to the arachnoidal granulations (Schmidt), the melanoblastomatosis to the leptomeningeal melanoblasts, while the association of glioma of the optic chiasm with the other lesions was pointed out long ago by Emanuel and

the relation of the cerebral lesions of tuberous sclerosis to those found in disseminated neurofibromatosis by Bielschowsky and Rose.⁶ How far can these normal structures involved be said to have a common embryological origin? In the beginning we must put some limit to our search. It is quite clear that all have arisen by progressive differentiation from the same ovum. It helps us little to go back even to the primary germ layers. The doctrine of the specificity of the germ layers is no longer tenable and it is generally accepted by embryologists that more than one primary germ layer can contribute to a normal structure of homogeneous histological structure when fully developed (Stone⁵⁰). With minor exceptions the nervous system, both central and peripheral, develops from the ectoderm along the medullary groove, and here we must distinguish early the medullary tube and the neural crest. The neural crest is a group of cells that lie in the angle between the medullary tube and the surface ectoderm. It has been shown by embryologists that cells from this formation migrate outward to form the dorsal roots of the spinal nerves, the dorsal root ganglia, the sympathetic nervous system, the medulla of the adrenal gland (Kohn²⁴) and other chromaffin structures. The dorsal root fibers are joined also by the prolongations of cells in the anterior horns of the spinal cord to form the spinal nerves (Held, Cajal). And it was further demonstrated by embryological experimentation, following the lead of Harrison²¹ that, if the neural crest be removed in amphibian larvae, not only is there no formation of dorsal spinal roots and sympathetic nervous system, but also that motor fibers of the anterior roots grow out without any accompanying Schwann cells. It is generally accepted, therefore, that the Schwann cells (also the Remak cells) are of neural crest origin with minor exceptions (olfactory nerves, Disse). The same cannot be said of many other structures covering the nervous system. The sheath cells of the Wagner-Meissner corpuscles cannot be proved in the same manner to arise from the neural crest since the lower vertebrate forms used for such experimentation do not have these corpuscles. They are believed, however, on embryological grounds to be analogous to Schwann cells (Klein²³). The cells of Langerhans also have not been proved to have such an origin, although the experiments of Dushane¹⁵ indicate that such may be the case in amphibia. The endoneurial-perineurial

tissue has been generally considered to arise *in situ* by condensation of the mesenchyme along the outgrowing nerves. An attempt was made by Harvey, Burr and von Campenhaut²² to prove that the leptomeninx, stated by Tarlov⁵⁵ to be continuous with the endoperineurium, arises from the neural crest, an idea previously expressed by Oberling³⁵ and supported by the presence in it of melanoblasts. And these attempts to prove an origin from the neural crest for the leptomeninges have found support (Raven⁴¹), yet no similar demonstration has been made for the endoperineurium. It is evident that the suppositions underlying the attempts to explain the association of these multiple tumors on the theory of a common embryological origin are not thoroughly substantiated. In particular the endoneurium-perineurium seems very unlikely to be of neural crest origin which makes all the more damaging the contention of certain authors (Penfield) that neurinomas arise from the perineurium. It is for this reason that Masson defends so vigorously the schwannian nature and origin of the neurinomas.

Pathology: The pathological arguments for the nervous nature of these lesions are largely by analogy. In the lesions of von Recklinghausen's disease structures are found that resemble Wagner-Meissner corpuscles (Brögli,¹⁰ Masson); the cell clusters of nevi are supposed to be an attempt to form the same structures (Masson³¹); also the palisading of the nuclei in neurinomas is interpreted as organoid production comparable to the supporting apparatus of the tactile corpuscles (Masson²⁰). On the other hand, Penfield³⁷ points out analogies of the structure of neurinomas to the perineurial tissues. He remarks that among the tumor cells are many fibrils that react like collagen and reticulin and concludes therefore that the cells must be fibroblastic. His assumption is that only fibroblasts can form reticulin and collagen, an assumption denied by Masson and unsupported by general histological data unless one gives a very wide interpretation to the term fibroblast, since it is generally recognized that many endothelial cells form argyrophilic substances that react to silver in a manner similar to reticulin. The fact that in the cells of these tumors delicate fibrils are found that stain blue with phosphotungstic acid hematoxylin and by methods that stain neuroglial fibrils has been used both as an argument for the fibroblastic nature

of the neurinomas (Penfield, Mallory) and for their neuroglial nature (Lhermitte). It is obviously an inconclusive argument since fibrils with the same staining reactions can be found in cells both of mesodermal and of neuro-epithelial origin, and in many other epithelial cells as well.

We will turn now to our own observations on pathological material. A comparison of our descriptions and microphotographs in the 2nd case (E.N.) seems to us conclusive that we are there dealing with the same alterations that have been so often described in cases of so-called hypertrophic interstitial neuritis (Wolf, Rubinstein and Burchell⁶¹). The whorls of cells about the axis cylinders have been interpreted by Boveri,⁸ Bielschowsky,⁵ Marie and Bertrand,²⁷ Souques and Bertrand,⁴⁷ Roussy and Cornil⁴² and others as schwannian proliferations. We feel that such an interpretation is unjustified in our case. We saw occasionally in the center of a whorl a distinct cell with abundant light cytoplasm, which stained yellow with van Gieson's and red by Masson's trichrome stain, and a vesicular nucleus (Fig. 33). Rarely a larger mass of apparently syncytial structure containing several nuclei with delicate cytoplasm and no reticulin was found (Fig. 38). These formations resembled exactly those described and illustrated by Marie and Bertrand.²⁷ But such formations had to be searched out with difficulty. There was no doubt that many of the whorls formed around myelinated nerve fibers, perhaps all of them, but we could see no good reason to suppose that they arose from Schwann cells. On the contrary, they seemed clearly to be continuous with and identical in structure with the endoneurium and perineurium. They were composed of elongated spindle shaped cells with dense scanty cytoplasm and wrinkled crenelated nuclei, interspersed by fibrils of collagen and reticulin. We can see no good reason to suppose that these whorls were not formed by the endoneurial tissue. If the formations in nevi and again in other peripheral tumors can be conceived to resemble Wagner-Meissner corpuscles, whose supporting cells are accepted to be of schwannian origin (Klein²³), these whorls as clearly resemble the corpuscles of Vater-Pacini which contain only a central column of schwannian cells and a laminated capsule of mesodermal cells.

It is furthermore remarkable that in many tumors the areas of whorls passed over by gradual transition into other areas with the

typical classical structure of neurinomas. If the whorls are of endoneurial origin, we can find no good reason to suppose that the neurinomatous formations are not also. In his classical study of experimental and spontaneous schwannomas Masson ²⁹ states that "in certain examples of generalized neurofibromatosis, studying nerves of apparently normal dimensions, several authors (Verocay, Pick and Bielschowsky) have seen microscopic tumors composed of Schwann cells in proliferation." We have perused carefully the studies which Nageotte and Masson have made of schwannian proliferations and we have repeated and confirmed their experiments; we have studied known schwannian proliferations in human amputation stumps; we have carefully studied the published observations of Verocay,⁵⁸ Pick and Bielschowsky,³⁹ and many others; we have studied numerous small neurinomas from both of our cases, and we have never been able to convince ourselves that any one of them arose from schwannian structures. They can be seen to arise within nervous funiculi, with no attachment to the perineurium, but whether from the Schwann sheaths or endoneurium we were never able to determine. We were, however, in the 1st case (B.S.) able to find several nodules in the spinal nerve roots of definitely neurinomatous structure arising unmistakably from the perineurium.

We were much more impressed by the resemblance of the tumors in our 2nd case (E.N.) to the ordinary leptomeningioma (Figs. 40, 29, 37). If we make abstraction of the nerve fibers which were contained in parts of them we fail to find any other differences. Also one of the leptomeningial tumors in this case had a structure identical with that of the acoustic tumor. The more we have studied the tumors from this remarkable case the more we have been impressed with the close relation between the neurinomas and leptomeningiomas; and the conviction has grown upon us that the Schwann cells, although they may undergo a slight hypertrophy secondary to the degeneration of nerve fibers in the tumor, have nothing to do with the tumor formation. This experience has shaken our faith in the schwannian origin of any case of hypertrophic interstitial neuritis. We see in the formations depicted by Roussy and Cornil ⁴² a proliferation of the Key-Retzius apparatus and, although in the case reported by Marie and Bertrand we can believe that there was some hypertrophy of the

schwannian apparatus such as we have also seen (Fig. 38), we believe such schwannian proliferation plays a purely secondary rôle.

In the study of our 1st case (B.S.), which is unquestionably one of generalized neurofibromatosis, we have found, and described above, typical schwannian hypertrophies secondary to the degeneration of nerve fibers, but rarely anything resembling tumorous proliferation (Figs. 5 and 6). After careful scrutiny of dozens of sections we could never find budding or branching of nerve fibers, which instead always ran in parallel bundles (Fig. 10), the only changes in them being degenerative. These bundles seemed to us to be entirely similar to those described by Bielschowsky⁶ in Boveri's case. The changes we observed in both the schwannian and Remak apparatus were essentially reactive or degenerative, rarely proliferative. On the other hand, the vast overgrowth of the nerves of this case seemed to us to be clearly of fibroblastic nature and we see no reason to consider it to be derived from any other tissue than the endoperineurial sheaths. This fibroblastic proliferation underwent a widespread liquefaction necrosis which by the accumulation of fluid dispersed the remaining cells widely. It is interesting to note the elongated bipolar cells of this tissue (Fig. 9), which were doubtless similar to those figured by Bruce and Dawson¹¹ in their case and interpreted by them as neurogenic cells which, forming in chains, were supposed to generate nerve fibers.

As a result of our observations we have become very skeptical of the schwannian theory of the origin and nature of any tumors of the peripheral nerves. Such publications as those of Stout,⁵¹ Martin and Déchaume,²⁸ Geschickter,²⁰ Stewart and Copeland,⁴⁹ or of Foot,¹⁸ advance the solution of this particular problem not at all. The schwannian theory seems to us to be more likely to explain satisfactorily the origin of such tumors as those described by Cohn,¹³ and by Stout,⁵² also Bergstrand,⁴ in the peripheral nerves, by Masson²⁹ in the palate, or by Cid¹² in the scalp; but when extended to the ordinary neurinomas we find the evidence unconvincing and it seems to us unlikely to become more convincing until better and more specific technical methods are available. The neural crest theory seems as little likely to prove fruitful. If we accept an origin from the neural crest for the leptomeninx the findings in our 2nd case (E.N.) indicate that it must be ex-

tended also to include the endoperineurium. There is to our knowledge no justification for such an extension.

But if we reject the schwannian hypothesis many questions remain to be answered. What of the association of all these various pathological alterations? What of the undoubted fact that tumors of the nerves have a distinctive structure not found in fibroblastomas elsewhere? What of the lesions in the brain? Many of the latter are composed of elongated cells with sausage shaped nuclei bearing such a close resemblance to malignant tumors of the peripheral nerves that they have been called a lemmoblastosis (von Sántha ⁶⁰). Antoni ² suggests that part of the neural crest tissue in such cases has been incorporated in the brain. Certainly if those cases that have tumors or malformations affecting both the neuraxis and peripheral nerves are to be explained on the basis of a maldevelopment, then the fault must involve both the medullary plate and the neural crest. And in such a case what is to prevent maldevelopment occurring in associated structures even if they are not derived embryologically from the neurectoderm? Speidel ⁴⁸ has shown that the proximity of a Schwann cell is necessary for the production of a myelin sheath by a peripheral axis cylinder, but that this stimulus alone is not sufficient; many of them remain unmyelinated even in the presence of a Schwann (Remak) cell if they do not have the proper central connections. If the neurectodermal structures are malformed, associated mesodermal structures, lacking the proper stimulus, will also not develop properly, and thus possibly also be liable to neoplastic change. The whole development of inductive embryology by Spemann and his school indicates that the orderly development of the body is due to a concatenation of mutually interacting forces, the derangement of any one of which will deviate the orderly unfolding of the rest. So the association of tumors of various structures may be explained on another basis than that of a common embryological derivation, and we see no reason why in generalized neurofibromatosis tumors and malformations should not simultaneously occur in structures of both neurectodermal and mesodermal derivation. This attitude toward the problem has already been suggested by Worster-Drought, Dickson and McMenemey, ⁶² and seems to us very reasonable. As for the distinctive structure of tumors of the peripheral nerves, certainly true in spite of the attempts of some pathologists

(Krumbein ²⁵) to belittle it, it does not necessarily argue in favor of a neurectodermal origin of the tumor cells but possibly only for a small degree of specialization from the fibroblasts of other connective tissues, just as certain cells of the general matrix of the leptomeninx, although derived from the general mesenchyme, have sufficiently specialized to form tumors with a distinctive structure.

SUMMARY AND CONCLUSIONS

1. Two cases of multiple tumorous proliferation of nervous and associated tissues have been described.

2. The cells of Schwann are believed to play a minor and secondary rôle in the production of the tumors of the peripheral nerves in these cases.

3. The origin of tumors of the peripheral nerves remains for us doubtful because the cells of the two possible sources (Schwann cells and endoneurium) form similar intercellular substances and specific staining or impregnation methods for identifying their cytoplasm have not been devised.

4. Although we began our study thoroughly impressed by the attractive schwannian hypothesis, we have been persuaded by our own observations that a schwannian origin of the common neurinomas and spindle celled malignant tumors of the peripheral nerves is very doubtful. The admission of this doubt leaves intact our admiration for Masson's brilliant demonstration of the neurectodermal origin of nevi and leaves us still able to accept as probable the neurectodermal origin of such tumors as that described in the palate by Masson and Panneton, or in the scalp by Cid, or even many others of unusual structure in the peripheral nerves (Brandes,⁹ Stout,⁵¹ Cohn,¹³ and Bergstrand⁴).

5. Because of the distinctive structure of the common circumscribed tumors of the peripheral nerves we do not favor the term *perineurial fibroblastoma* but believe it better, if the time honored *neurinoma* will not do, to use for them a more distinctive term such as *neurilemoma*, as proposed by Stout.⁵²

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DESCRIPTION OF PLATES

PLATE I

FIG. 1. Case 1, B.S. Dorsal spinal root at the eleventh thoracic segment.
Hematoxylin-eosin stain. $\times 60$.

FIG. 2. Case 1, B.S. Anterior spinal root at the eleventh thoracic segment.
Hematoxylin-eosin stain. $\times 60$.

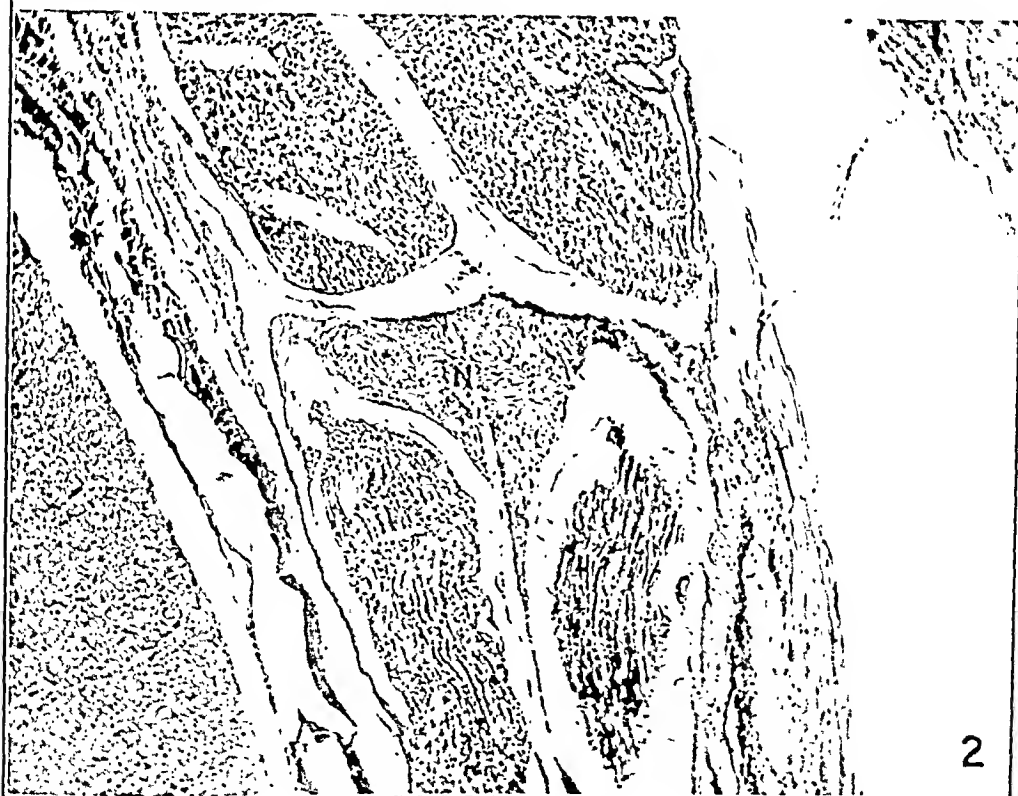
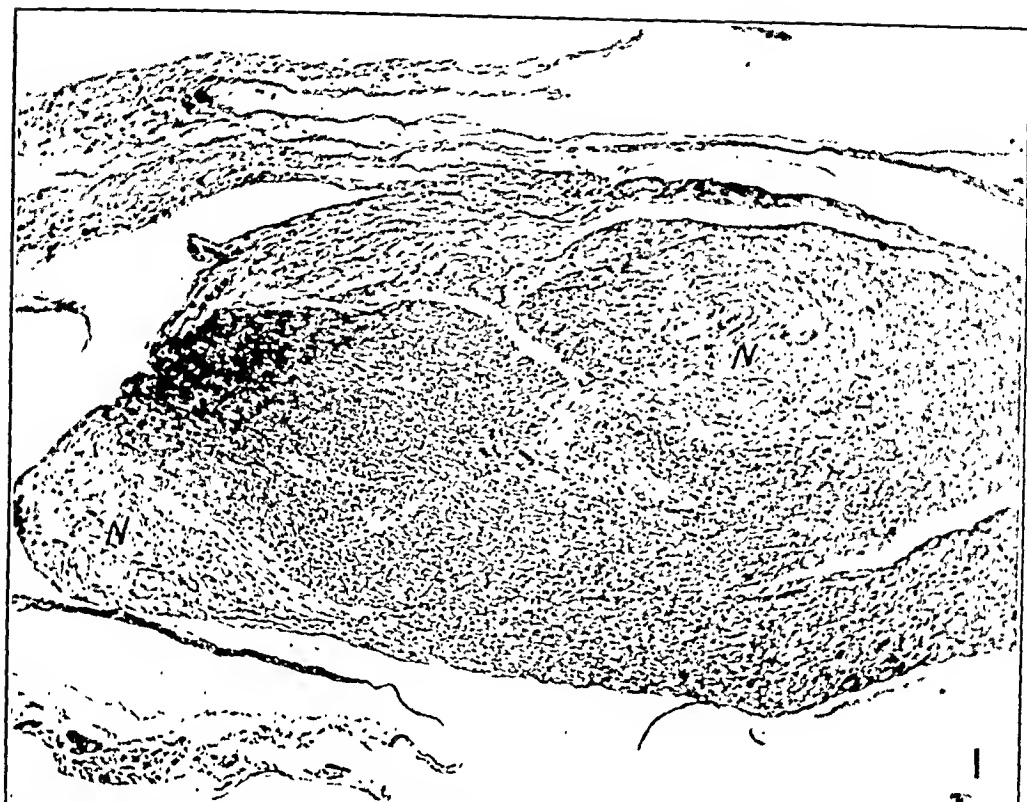


PLATE 2

FIG. 3. Case 1, B.S. Lumbar plexus. Van Gieson's stain. $\times 700$.

FIG. 4. Case 1, B.S. Lumbar plexus. Van Gieson's stain. $\times 700$.

FIG. 5. Case 1, B.S. Lumbar plexus. Masson's trichrome stain. $\times 600$.

FIG. 6. Case 1, B.S. Lumbar plexus. Masson's trichrome stain. $\times 600$.

B = Buengner cords; F = fibroblasts; R = Remak bands; S = schwannian proliferations (in Fig. 5 in longitudinal section, in Fig. 6 in cross section).

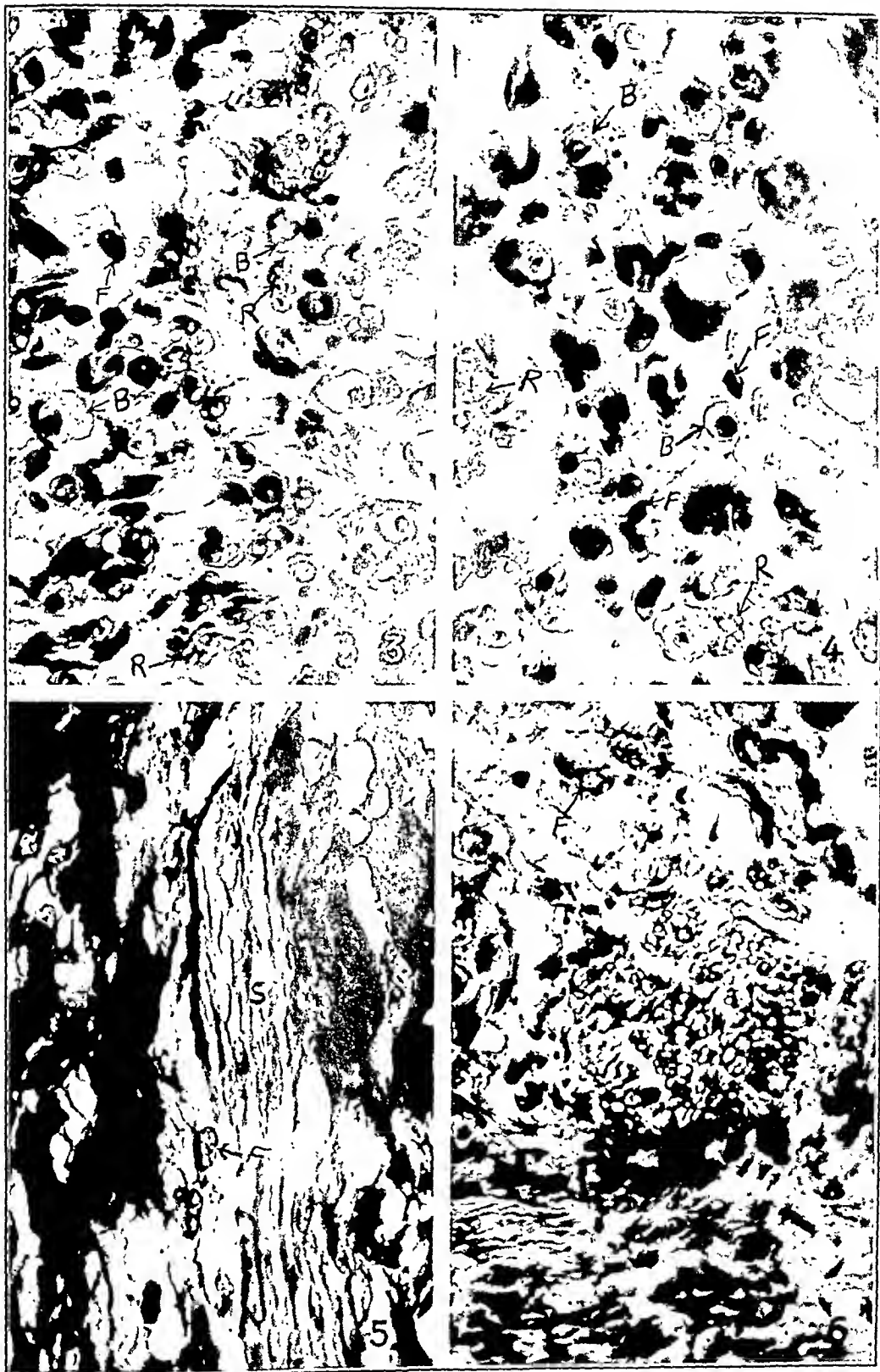


PLATE 3

FIG. 7. Case 1, B.S. Tumor of the first cervical nerve. Masson's trichrome stain. $\times 600$.

FIG. 8. Case 1, B.S. Lumbar plexus. Note the proliferation of schwannian nuclei about a myelinated nerve fiber. Hematoxylin-eosin stain. $\times 600$.

FIG. 9. Case 1, B.S. Lumbar plexus, showing fibroblasts in a degenerated area. Hematoxylin-eosin stain. $\times 600$.

FIG. 10. Case 1, B.S. Lumbar plexus. Bodian's method. $\times 300$.

B = Buengner cord; F = fibroblast; M = myelinated nerve fiber; S = nuclei of Schwann cells; U = unmyelinated nerve fibers.

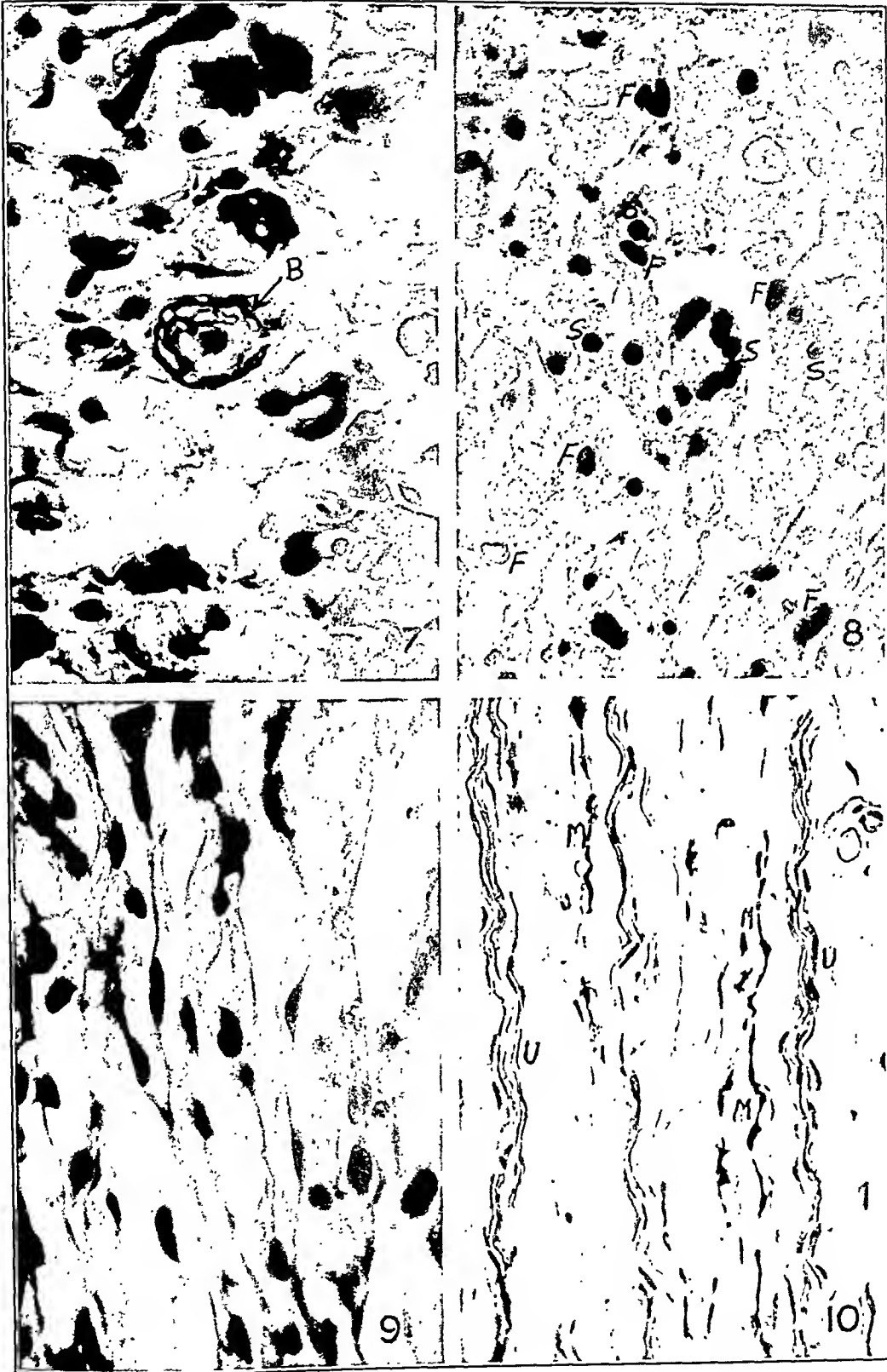


PLATE 4

FIG. 11. Case 1, B.S. Lumbar plexus, various stages of degeneration. Freeman's method. $\times 600$.

FIG. 12. Case 1, B.S. Lumbar plexus, various stages of degeneration. Van Gieson's stain. $\times 600$.

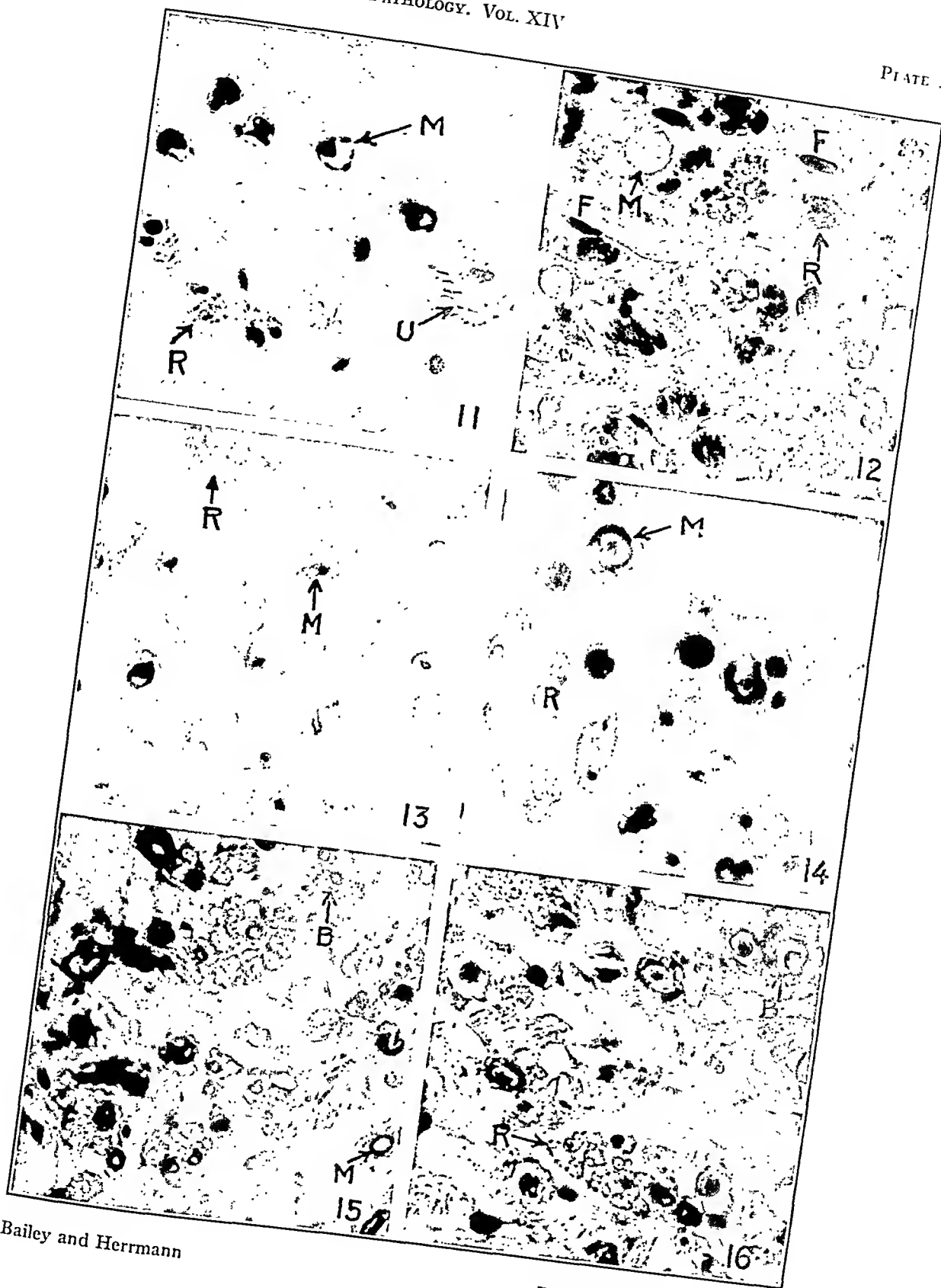
FIG. 13. Case 1, B.S. Lumbar plexus, various stages of degeneration. Freeman's method. $\times 800$.

FIG. 14. Case 1, B.S. Lumbar plexus, various stages of degeneration. Weil's method. $\times 700$.

FIG. 15. Case 1, B.S. Lumbar plexus, various stages of degeneration. Mason's trichrome stain. $\times 700$.

FIG. 16. Case 1, B.S. Lumbar plexus, various stages of degeneration. Mucicarmine stain. $\times 700$.

B = Buengner cord; F = fibroblasts; M = myelinated nerve fibers; R = Remak bands; U = unmyelinated nerve fibers.



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Rôle of the Cells of Schwann

PLATE 5

FIG. 17. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Freeman's method. $\times 800$.

FIG. 18. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Weigert-Pal. $\times 700$.

FIG. 19. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Foot's method. $\times 600$.

FIG. 20. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Laidlaw's method. $\times 600$.

FIG. 21. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Van Gieson's method. $\times 600$.

FIG. 22. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Masson's trichrome method. $\times 700$.

R = Remak bands.



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Rôle of the Cells of Schwann

PLATE 6

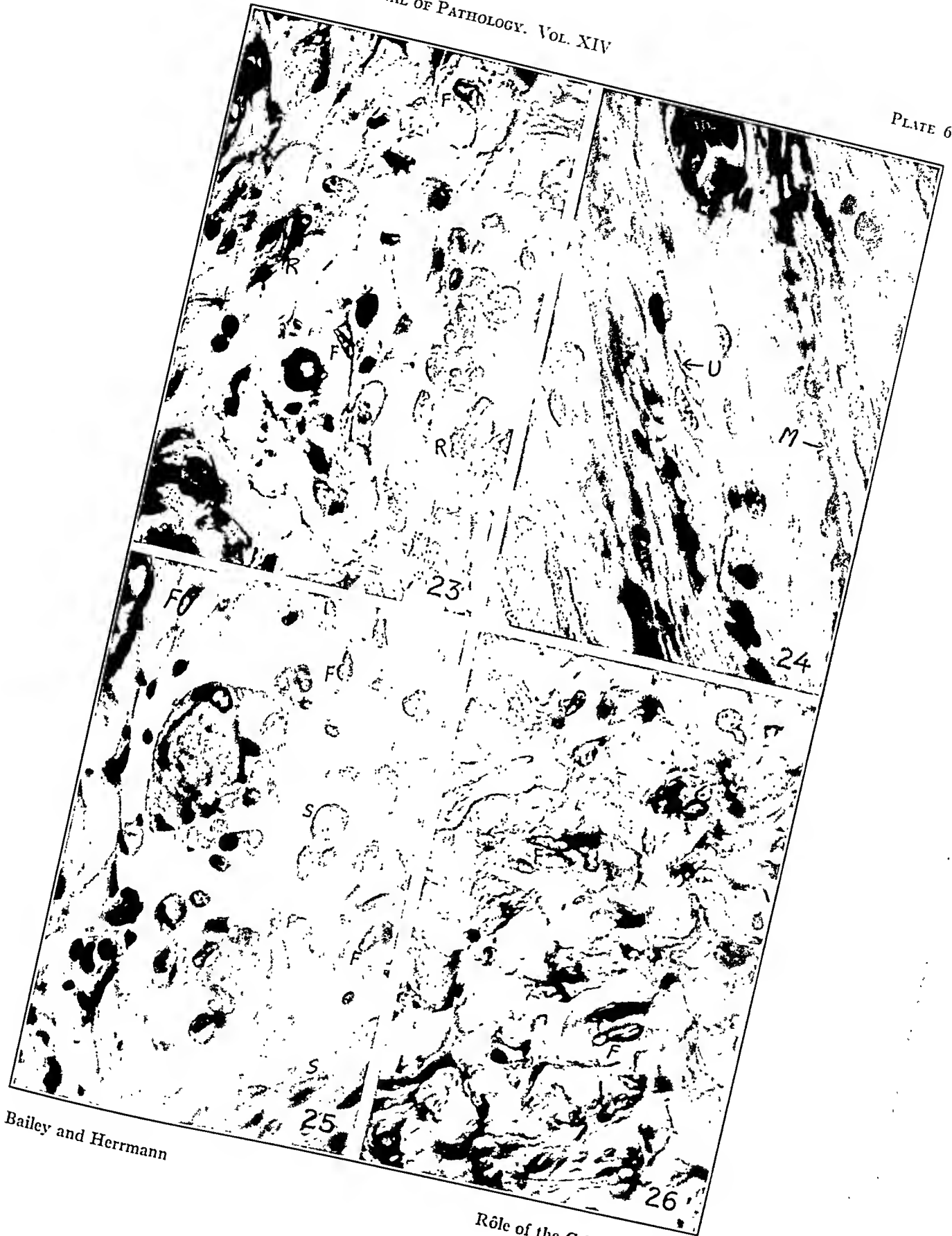
FIG. 23. Case 1, B.S. Sympathetic trunk. Hematoxylin-eosin stain. $\times 600$.

FIG. 24. Case 1, B.S. Dorsal spinal root. Hematoxylin-eosin stain. $\times 600$.

FIG. 25. Case 1, B.S. Sympathetic trunk. Hematoxylin-eosin stain. $\times 600$.

FIG. 26. Case 1, B.S. Sympathetic trunk, from an area of pure fibroblastic proliferation. Hematoxylin-eosin stain. $\times 600$.

F = fibroblasts; M = myelinated nerve fiber; S = Schwann cell; R = Remak band; U = unmyelinated nerve fiber.



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Rôle of the Cells of Schwann

PLATE 7

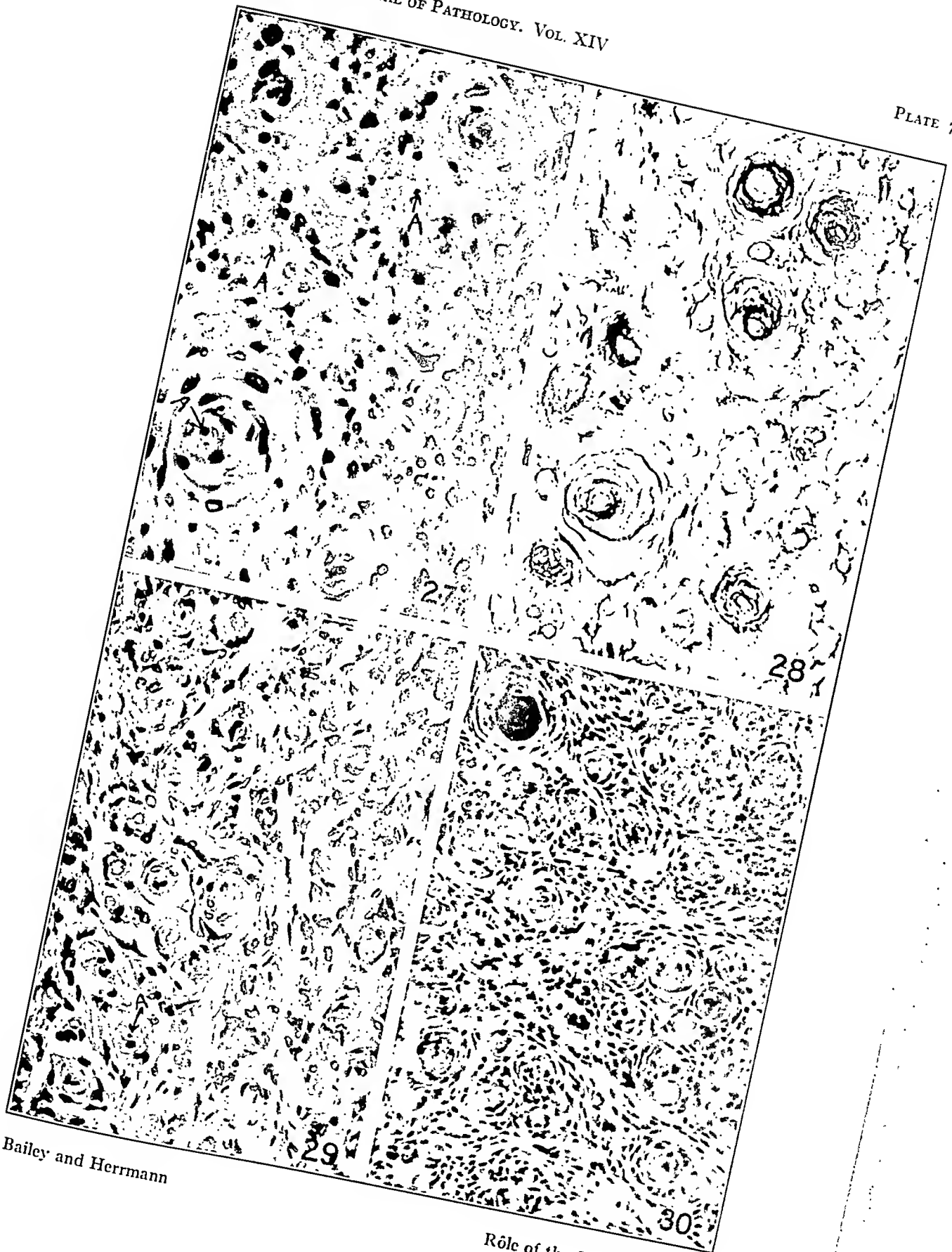
FIG. 27. Case 2, E.N. Left oculomotor nerve. Hematoxylin-eosin stain. $\times 300$.

FIG. 28. Case 2, E.N. Left oculomotor nerve. Perdrau's method. $\times 300$.

FIG. 29. Case 2, E.N. Right vagus nerve. Hematoxylin-eosin stain. $\times 250$.

FIG. 30. Case 2, E.N. Meningeal tumor at sixth cervical level. Hematoxylin-eosin stain. $\times 150$.

A = axis cylinders.



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Rôle of the Cells of Schwann

PLATE 8

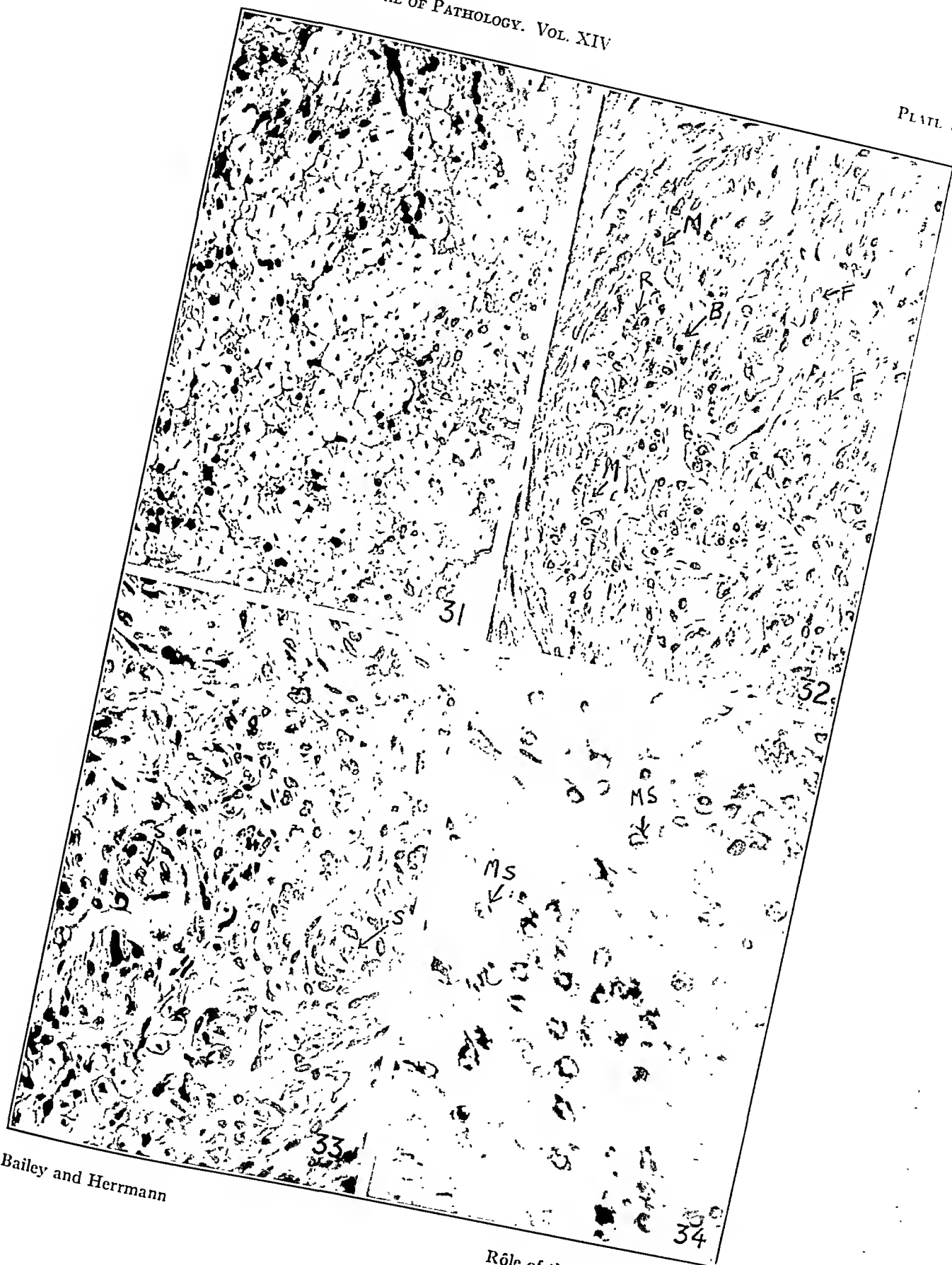
FIG. 31. Case 2, E.N. Normal funiculus from the dorsal root of the eleventh thoracic nerve. Hematoxylin-eosin stain. $\times 300$.

FIG. 32. Case 2, E.N. Degenerated funiculus from the same nerve root. Hematoxylin-eosin stain. $\times 300$.

FIG. 33. Case 2, E.N. Tumor of right oculomotor nerve. Hematoxylin-eosin stain. $\times 300$.

FIG. 34. Case 2, E.N. Right oculomotor nerve. Loyez's method. $\times 300$.

B = Buengner cord; F = fibroblasts; MS = persisting myelin sheaths within whorls; S = nuclei of Schwann cells within whorls from which axis cylinders have disappeared; R = Remak bands.



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Rôle of the Cells of Schwann

PLATE 9

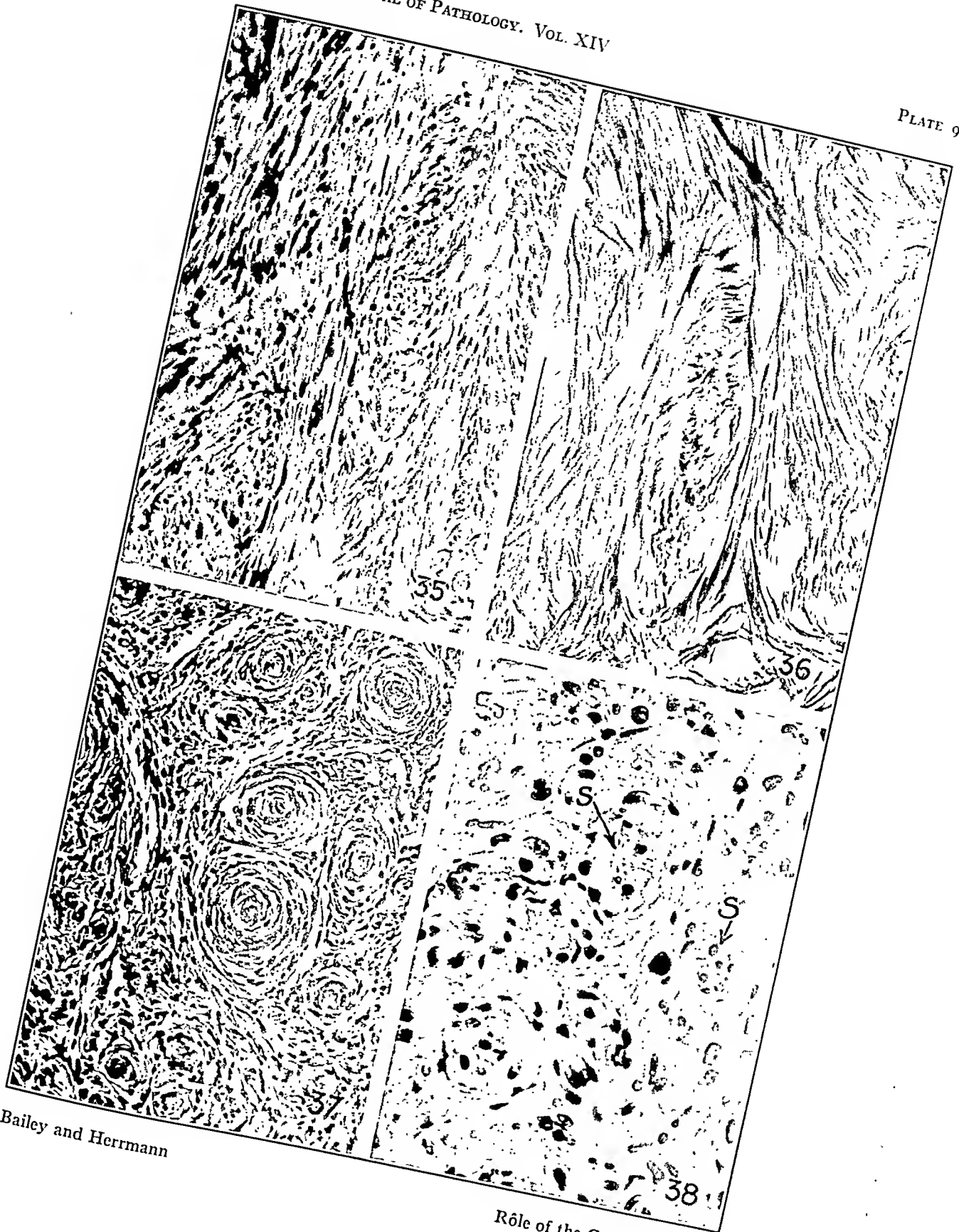
FIG. 35. Case 2, E.N. Left acoustic tumor. Hematoxylin-eosin stain. $\times 150$.

FIG. 36. Case 2, E.N. Left acoustic tumor. Perdrau's method. $\times 150$.

FIG. 37. Case 2, E.N. Right acoustic tumor. Hematoxylin-eosin stain. $\times 150$.

FIG. 38. Case 2, E.N. Right oculomotor nerve. Hematoxylin-eosin stain.
 $\times 300$.

S = schwannian proliferations.



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Rôle of the Cells of Schwann

PLATE 9

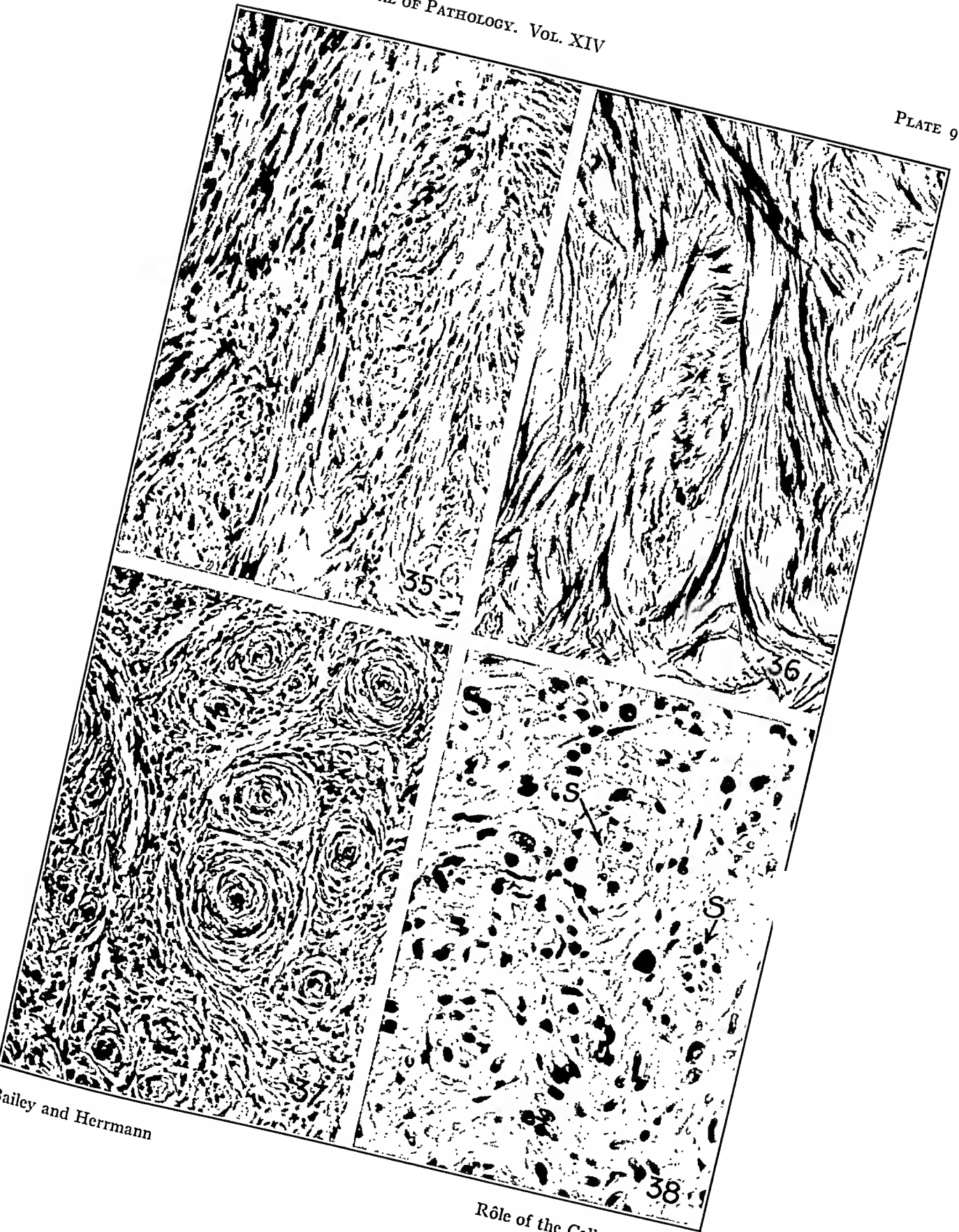
FIG. 35. Case 2, E.N. Left acoustic tumor. Hematoxylin-eosin stain. $\times 150$.

FIG. 36. Case 2, E.N. Left acoustic tumor. Perdrau's method. $\times 150$.

FIG. 37. Case 2, E.N. Right acoustic tumor. Hematoxylin-eosin stain. $\times 150$.

FIG. 38. Case 2, E.N. Right oculomotor nerve. Hematoxylin-eosin stain.
 $\times 300$.

S = schwannian proliferations.



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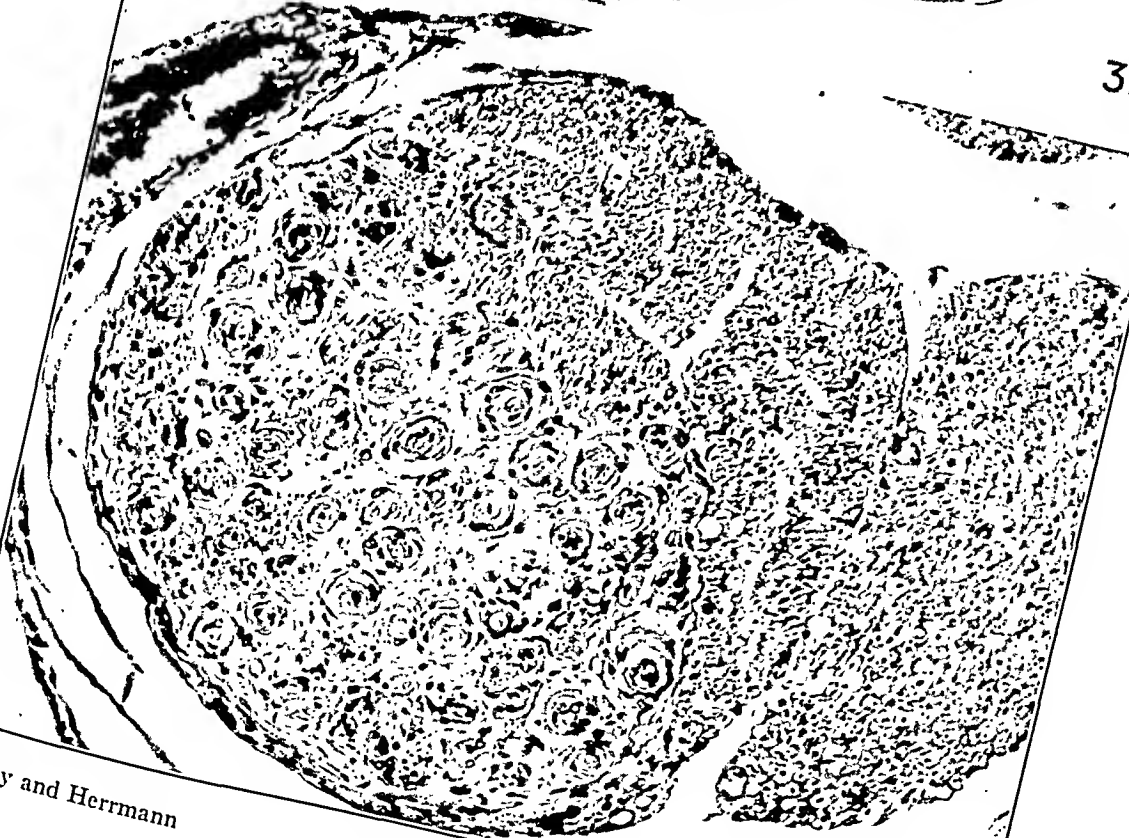
Rôle of the Cells of Schwann

PLATE 10

FIG. 39. Case 2, E.N. Cross section of the spinal cord at the fourth lumbar segment. Note the extension of the tumor of the posterior root into the left posterior horn. Hematoxylin-eosin stain. $\times 9$.

N = neurinoma.

FIG. 40. Case 2, E.N. Cross section of nerve root indicated by arrow in Fig. 39. Hematoxylin-eosin stain. $\times 150$.



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Rôle of the Cells of Schwann

PLATE 10

FIG. 39. Case 2, E.N. Cross section of the spinal cord at the fourth lumbar segment. Note the extension of the tumor of the posterior root into the left posterior horn. Hematoxylin-eosin stain. $\times 9$.

N = neurinoma.

FIG. 40. Case 2, E.N. Cross section of nerve root indicated by arrow in Fig. 39. Hematoxylin-eosin stain. $\times 150$.



THE PATHOLOGY OF GRANULOMA VENEREUM *

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Granuloma venereum, a disease widespread in tropical and subtropical countries, is best defined as an infectious granuloma of the pudenda. The question whether it is transmitted by sexual contact and should be considered a venereal disease is still undecided, but the experimental work of DeMonbreun and Goodpasture makes its traditional venereal nature appear at least doubtful.

In a previous study we discussed the incidence of granuloma venereum in various parts of the United States and, to some extent, were able to confirm Harris' statement that the disease seems to travel from the large seaports toward the inland sections along the great waterways. Supplementing Fox's report, we have collected 251 cases of granuloma venereum from the literature. This figure, however, is certainly not indicative of the true incidence of the infection in the United States, nor is our recent report of 294 cases observed over a period of 5 years in New Orleans a true gauge of the incidence of the disease in that community. Lack of cooperation on the part of afflicted patients, who generally belong to the lowest social strata, prevents exact diagnosis in many instances.

During studies of the various manifestations of the disease we have had occasion to examine not only surgically removed specimens and material from autopsied cases, but also, through the cooperation of the hospital staff, numerous biopsy specimens. It is the histopathological findings in this material that we wish to discuss in this commentation.

PATHOLOGICAL ANATOMY

The numerous classifications of the various lesions of granuloma venereum are based principally on their morphological character without consideration of their underlying pathogenesis. In our clinical study we have adopted, in a somewhat modified form, the purely descriptive terms applied to the lesions by Halty and have classified these as nodular, serpiginous, necrotic, hyper-

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trophic and cicatricial. From the standpoint of their pathological variations, however, we differentiate three groups of lesions: (1) those caused primarily by the infectious agent; (2) those caused principally as a result of a peculiar tissue reaction of the host to the infection; and (3) those caused by complications following the infection.

The nodular and serpiginous types of lesions, which were observed in 157 or 53.4 per cent of our cases, belong to the first pathological group. The nodular lesions are usually only the beginning stage of infection and undergo further changes which lead to development of the serpiginous ulcer, the most common and most typical manifestation of the disease. The lesion is characterized by a soft, easily bleeding granulation tissue which breaks through the epithelial lining of the skin and mucous membrane and shows a remarkable tendency towards superficial spread along the moist folds of the inguinal and pudendal regions (Manson). These lesions are only a few mm. deep and usually show very little suppuration. The exudate is serosanguineous, contains the infectious agent and spreads the disease by auto-inoculation. The rapidity with which the infection spreads varies considerably and healing may be observed in some portions of the lesion while other parts show continuous encroachment upon sound tissue. During the course of the disease considerable areas of the skin and mucous membranes are usually covered with these ulcerative lesions, with resultant severe anatomical mutilation of the genitalia and the perineum. The healing process is slow and the scars produced are atrophic with partial depigmentation of the skin and permanent loss of pubic hair.

The second group of pathological manifestations is the result of a peculiar host reaction to the infectious agent leading to hypertrophic and keloid-like lesions. These were present in 82 or 27.9 per cent of our cases. The surfaces of the hypertrophic lesions may be compared to the relief map of a mountainous country, with depressions between areas of piled up "mountains of tissue" (Harris). In consistence the lesions are firm and rather elastic, the overlying skin usually showing scars of previous ulcerations. There is often very little difference between this type of lesion and the true cicatricial or keloid-like lesion. In the latter there is an apparent overproduction of firm indolent tissue which replaces the

ulcerations. Our attention was first called to this type of lesion by complaints of patients that the scars from previous ulcerations were inclined to spread with gradual involvement of healthy parts in the keloid-like process. There is a distinct difference between this "spreading" type of scar and the usual atrophic and shrunken scar seen following ulcerative lesions. Our suspicion that we were dealing not with a healed stage of the disease but with a progressive lesion was further confirmed by the fact that histological examination showed the presence of Donovan bodies in the small nests of inflammatory cells embedded in the dense collagenous fibrous tissue obtained from such scars. Both the hypertrophic and the cicatricial (keloid-like) lesions show an excessive fibroblastic response of the host to the infection, usually developing rather early and inclined to progression. We believe that this lesion, observed rather commonly in the negro race, is due to an unexplainable constitutional peculiarity of the patient and not to chronic lymphatic obstruction, as claimed by Daniels. A further difference between the hypertrophic and keloid-like variety of granuloma venereum is found in the amount of intercellular collagenous substance present. In the hypertrophic form the amount is relatively small as compared to the mass of newly formed fibrocytes, while in the cicatricial lesion it is abundant.

The third group of pathological manifestations of granuloma inguinale consists of the lesions that occur as complications of the primary infection. The most frequent of such is secondary infection with an aerobic or anaerobic pyogenic or saprophytic genus. The onset of a virulent secondary infection is usually characterized by the appearance of toxic constitutional symptoms which are completely absent in the uncomplicated forms of granuloma venereum and by progression of the ulcerative process with the production of deep severe necrosis of soft tissues and even bone. During this stage Donovan bodies are generally not demonstrable, but there is an abundant mixed flora of secondary bacterial invaders. Here, the healing process results in severe mutilation of the genitalia with usual permanent impairment of their function. Fifty-five cases or 18.7 per cent of our series showed such deep ulcerations with necrosis and phlegmonous extension into the surrounding tissue. Two presented extragenital lesions, 1 case showing deep necrosis of the mouth and the structures of the neck with

secondary bronchopneumonia, the other a phlegmon of the gluteal region.

HISTOPATHOLOGY

Fifty-six biopsies and 3 autopsies furnished the material for histopathological studies. In 3 cases a series of biopsies was obtained, enabling study of the evolution of the disease. Tissues were fixed in formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin, Wright's Gram's, and Giemsa's stains, and also by Mallory's aniline blue collagen stain.

In the very early or nodular type of granuloma venereum the epithelial lining of the skin does not appear to be interrupted, but there is distinct hypertrophy of the epithelium with offshoots from the papillae into the subcutaneous tissue (Gage). There is some edema in the papillary layers and infiltration of the corium of the skin, with polymorphonuclear leukocytes, eosinophiles and large monocytes (endothelial cells); these show no characteristic arrangement but seem embedded in a rather edematous matrix. With bacteria-revealing stains, numerous intracellular inclusion bodies — the so-called Donovan bodies — can be noted in the plasma of the large endothelial cells. On account of the pressure caused by infiltration the epithelium of the affected areas becomes thinned out and atrophic, exudate seeping through before the epithelial continuity is actually interrupted. Rapid proliferation of capillaries in the area of infiltration marks the beginning of the development of granulation tissue, which soon breaks through the epithelial lining of the skin to form the typical serpyiginous lesions. Plasma cells, diffuse and in small groups, become increasingly prominent with progression of the lesion, and the leukocytes, which previously seemed to be the most important primary cellular response, are now found only at the surface. Large endothelial cells with numerous intracellular Donovan organisms are profuse between the capillary loops of the granulation tissue and are probably identical with the large foam cells described by Goldzieher and Peck as characteristic of this stage of the disease.

As the healing progresses, fibrocytes, which primarily were only sparsely scattered between the capillaries of the granulation tissue, become more abundant and, from the epithelial islands which have remained intact during the process of granulation tissue formation,

re-epithelialization of the surface begins. The scar tissue that repairs the serpiginous ulcer of granuloma inguinale usually shows a narrow epithelial lining with loss of all special structures of the skin and a moderate degree of subepithelial fibrosis. In the deep ulcerative processes, extensive necrosis with suppuration and phlegmonous extension may be noted. At the bottom of the necrotic areas there is sometimes fibrosis with interspersed nests of plasma cells and monocytes containing Donovan bodies. Bacterial stains reveal an abundant flora including *Borrelia vincenti* and fungi. Histological examination of the hypertrophic and the keloid-like lesions reveals marked fibrosis with numerous small nests of plasma cells and endothelial cells. The epithelium appears normal in thickness or shows slight hyperplasia. The walls of the larger vessels are thickened and their lumens narrowed. The lymph vessels are sometimes slightly dilated, but they are never the site of inflammatory changes as seen in elephantiasis caused by lympho-granuloma inguinale. The collagenous substance is extremely abundant in the hypertrophic cicatricial lesions. In the small collections of inflammatory cells Donovan bodies can be found, thus giving evidence of the activity of the lesion.

THE CAUSAL AGENT

Although granuloma venereum has been known for over half a century, its causal agent has not yet been definitely established. Formerly identified with lues (Maitland, MacLennan), rhinoscleroma (Goodman), tuberculosis (LeDantec), and various other infections, since 1904 granuloma venereum has been definitely linked with an organism described by Donovan as Piroplasma and considered by him protozoal in nature. Martini in 1913, and Aragao in 1917, cultivated this organism on Sabouraud's medium and Aragao gave it the name "Schizomycete kalymmatogranulomatis." Castellani and Mendelson succeeded in growing an encapsulated bacillus that showed a close morphological relation to the Donovan organism and which was identified culturally as belonging to the group of *Aerobacter aerogenes*, genus *Encapsulatus*, Castellani and Chalmers. However, they do not believe this to be the causal organism. Aragao denied the identity of any bacillus belonging to the group of *Klebsiella* with the *Calymmatobacterium*, believing that the latter has never been cultivated. Organisms not

definitely classified but showing characteristics similar to the ones obtained by Castellani were grown by Poindexter, and by Goldzieher and Peck. DeMonbreun and Goodpasture confirmed Castellani's findings by growing organisms belonging to the aerogenous group from human lesions. Although they produced the disease by injecting human material into monkeys, they failed to do so with cultures of organisms obtained from such material. Campbell, in criticising the work of McIntosh, states very emphatically that, to date, no lesions have been produced with organisms cultivated from venereal granuloma. In the past year, Menon and his co-workers have critically analyzed the bacterial flora present in venereal granuloma. In addition to the Donovan organism, they have noted in human cases various spirochetes and fusiform bacilli, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Corynebacterium diphtheriae* and numerous types of staphylococci. These Indian investigators have succeeded in isolating the Donovan organism in pure culture from 12 out of 14 cases, and believe it to be related to *A. aerogenes*. Its inoculation in young rats and mice resulted in the production of distinct pathological lesions. Although the majority of recent authors seem to agree that an organism belonging to the *Klebsiella* group can be recovered from a large percentage of lesions present in granuloma inguinale, its etiological significance is still debated.

We have been able to observe the Donovan organism in practically all tissue sections stained by Wright's method in 60 to 80 per cent of smears obtained from the lesion. The nucleus of the capsulated body resembles a small curved bacillus, shows one or two terminal swellings simulating polar bodies and may, therefore, readily appear as "diplococcoid bodies" (Randall, Small and Belk). In acute fulminating lesions the majority of the organisms are not encapsulated and can easily be recognized extra- and intracellularly. In the large mononuclear cells they may fill vacuolar spaces in small clumps or clusters, or may be present in such numbers as to obliterate completely the outline and structure of the cells. We have regularly found plastin bodies, as described by Goldzieher and Peck, and they have been of considerable help in diagnosis, although we do not understand their significance. Our attempts to cultivate an organism from the human lesions have been successful in 8 out of 11 cases. The organism, similar to that

isolated by DeMonbreun and Goodpasture, belongs to the aerogenes group, but we have failed, so far, to produce any pathological lesions in laboratory animals comparable with the human lesion.

SUMMARY AND CONCLUSIONS

1. The pathology of granuloma inguinale has been studied in a series of 294 cases observed over 5 years at the State Charity Hospital of Louisiana at New Orleans.

2. The typical manifestations of the disease embrace nodular lesions and serpiginous ulcerations, which have a tendency to spread along the moist folds of the pudendal region, healing with the formation of atrophic scars.

3. Atypical manifestations are produced by unexplained increased fibroblastic reaction of the host leading to hypertrophic and cicatricial (keloid-like) lesions, which must be considered active stages of the infection.

4. Secondary infection produces serious ulcerative necrotic lesions which may severely mutilate the infected parts and give rise to sepsis and toxemia.

5. Histopathological study of biopsy material and tissue obtained at autopsy reveals that the stage of infiltration is quickly followed by the stage of granulation, during which the epithelial lining of the skin or mucous membrane is perforated by a vascular granulation tissue. Donovan organisms can be demonstrated in the infected tissue during all stages of the infection.

6. Search for a causal agent has resulted in the isolation of an organism belonging to the *Klebsiella* group. Inoculation of various laboratory animals with this organism has failed to incite lesions comparable to the disease in the human, although such lesions have been reported as produced by inoculation of material derived from human cases.

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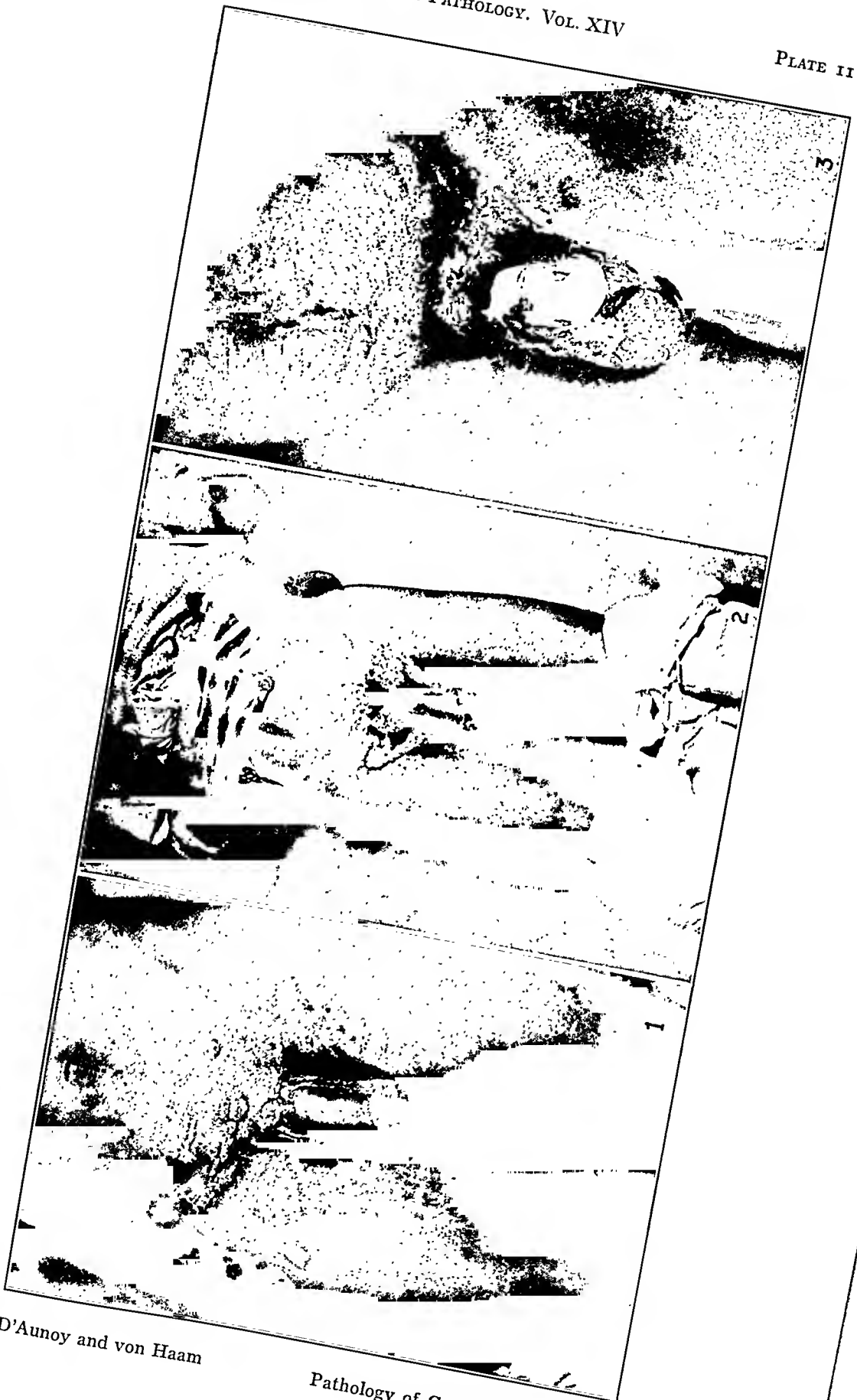
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DESCRIPTION OF PLATES

PLATE II

- FIG. 1. Male negro, aged 25 years. Multiple nodular lesions of 3 weeks duration.
- FIG. 2. Male negro, aged 32 years. Bilateral serpiginous ulcers of 4 months duration.
- FIG. 3. Male negro, aged 37 years. Cicatricial lesion with progressive mutilation of penis and scrotum of 3 years duration.



D'Aunoy and von Haam

Pathology of Granuloma Venereum

PLATE 12

FIG. 4. Negress, aged 28 years. Hypertrophic lesion involving labia and mons veneris of 1 years duration.

FIG. 5. Negress, aged 52 years. Deep ulceration with necrosis of entire perineum of 8 months duration.

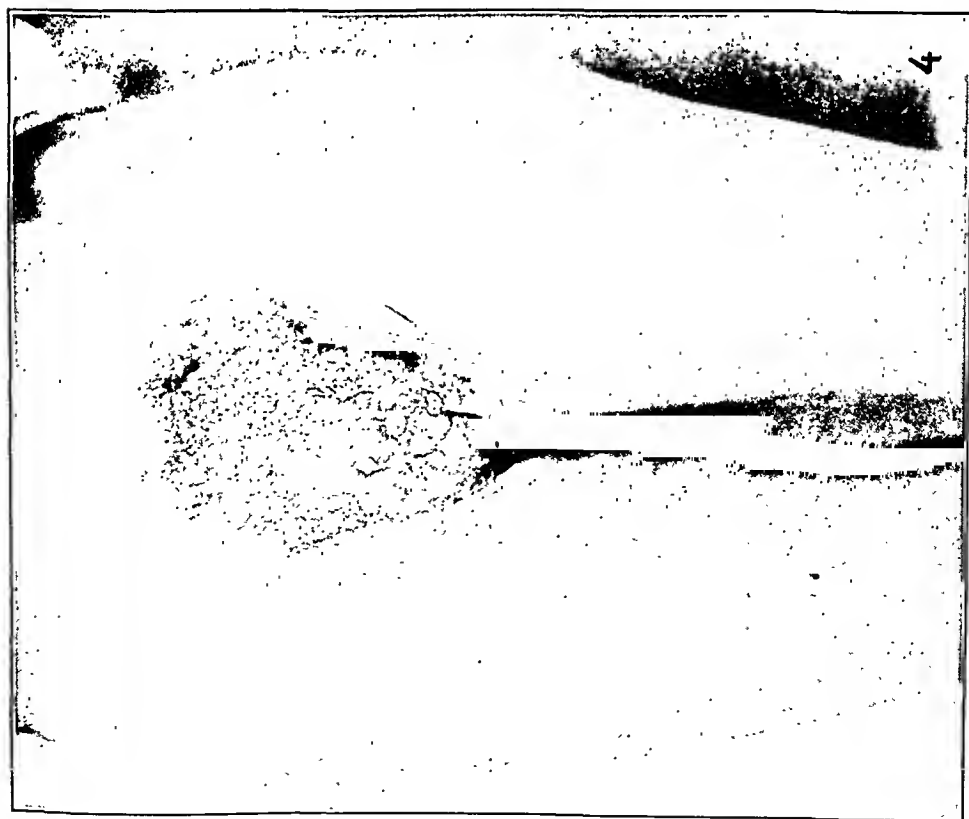
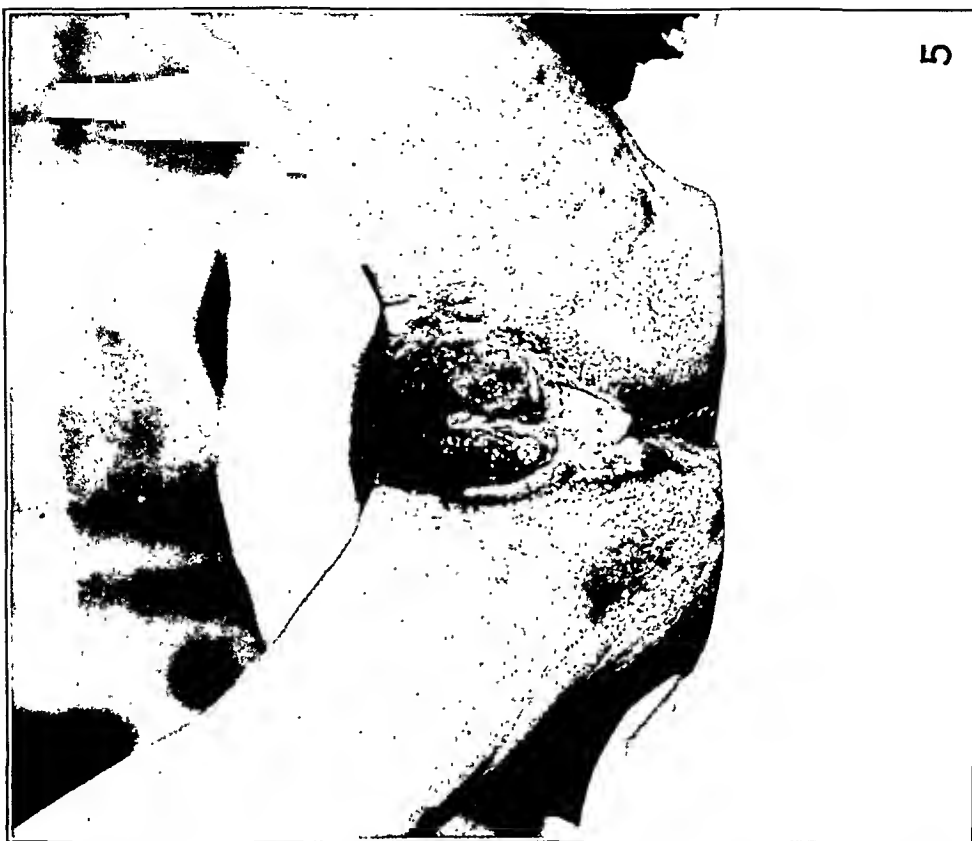


PLATE 13

- FIG. 6. Section through a nodular lesion showing initial proliferation of epithelium with subepithelial infiltration.
- FIG. 7. Section through the margin of a serpiginous ulceration showing atrophy of the epithelium with marked edema and exudation.
- FIG. 8. Section through the same lesion 3 weeks later showing development of a marked vascular granulation tissue.
- FIG. 9. Section through a cicatricial lesion showing numerous active foci of the infection embedded in collagenous tissue.

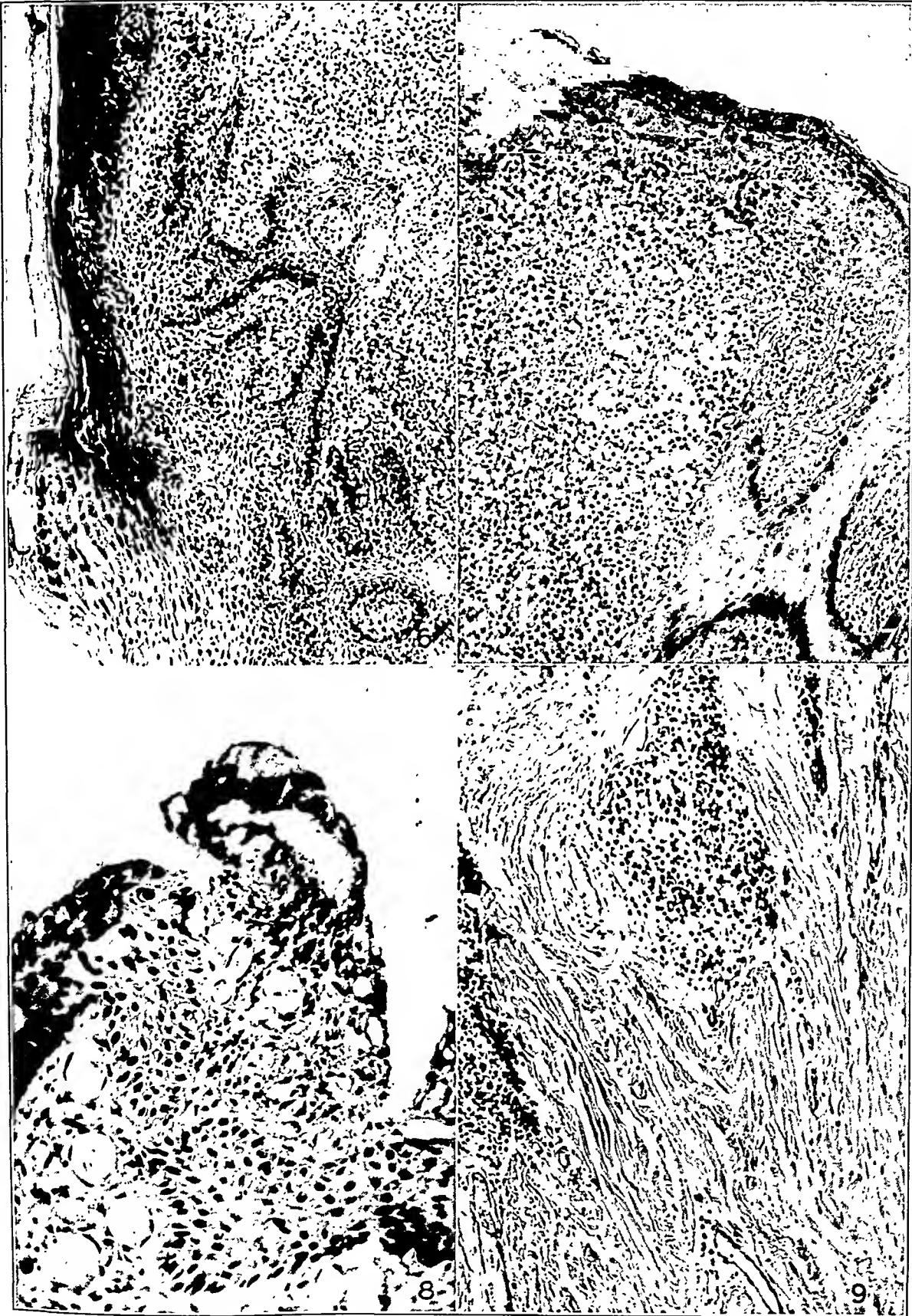
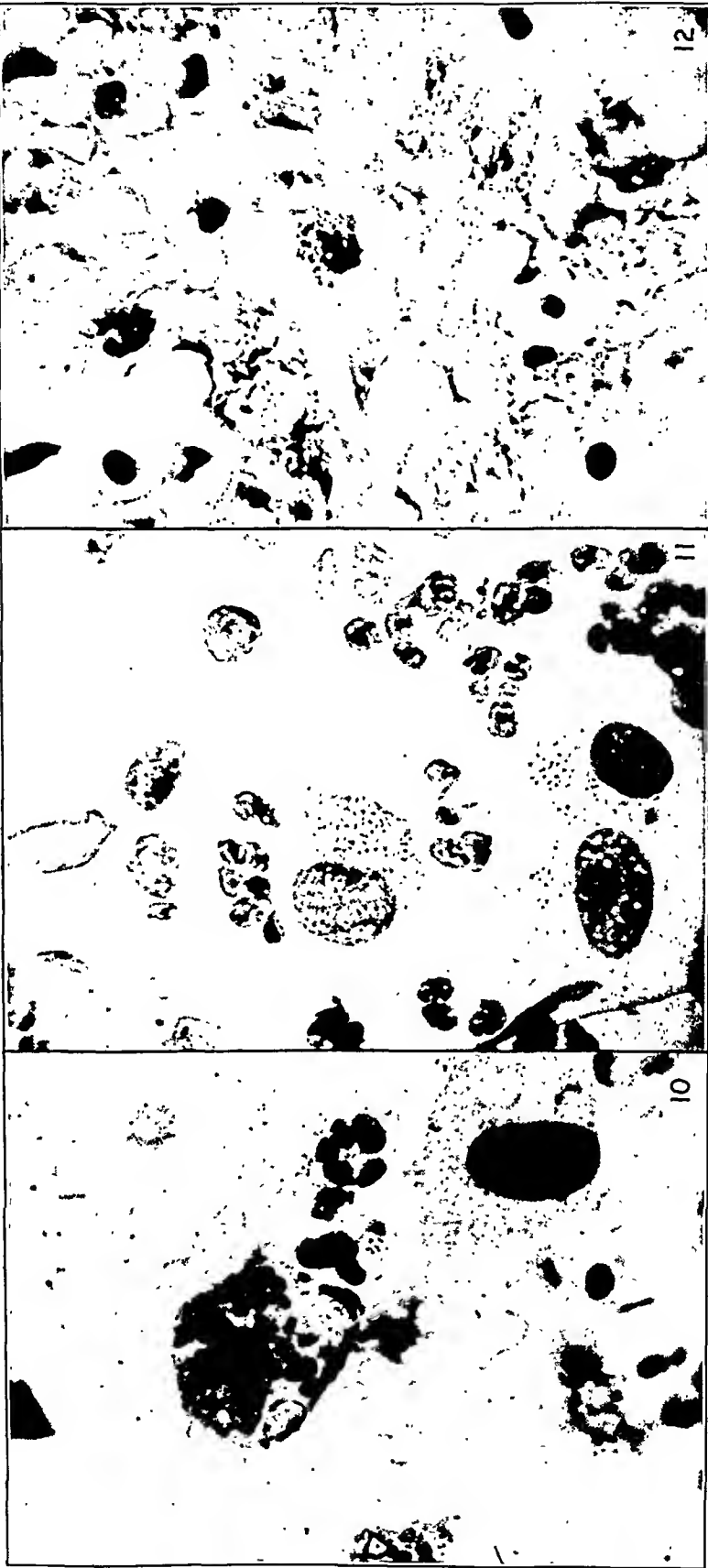


PLATE 14

- FIG. 10. Smear from a serpiginous ulcer stained with Wright's stain. Numerous Donovan bodies are present in the plasma of a large monocyte.
- FIG. 11. Smear from an acute ulcerative lesion stained with Wright's stain showing numerous non-encapsulated organisms growing in the plasma of monocytes.
- FIG. 12. Section through a cicatricial lesion stained with Wright's method demonstrating numerous encapsulated and non-capsulated organisms in the plasma of an endothelial cell.





PNEUMOCONIOSIS AND PULMONARY CARCINOMA *

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The increasing prominence of pneumoconiosis and the more common recognition of primary pulmonary carcinoma have led to a large number of current reports of tumor development in the lungs of individuals who have been exposed to industrial dusts. The assumption has followed that inhaled dust plays an important rôle in the etiology of carcinoma.

Much notoriety, even in this country, has attended the high incidence of pulmonary carcinoma in men working in the mines in Schneeberg, Germany, and St. Joachimstal, Czechoslovakia.¹⁻³ The etiology of this type of carcinoma, although obscure, was for some time attributed to the dust these miners inhaled. In a recent critical review of these cases, however, Saupe⁴ concludes that the newgrowth is due to radioactive substances in the air of these mines rather than to the inorganic dust. This interpretation is not generally recognized in the numerous publications that cite these cases as a foundation for their thesis that inhaled dust is a causative factor in the development of primary pulmonary carcinoma.

Many cases of carcinoma of the lung in persons exposed to inhalation of different dusts in occupation have been described in the recent literature. They are cited in the following table:

AUTHOR	CASES REPORTED	AUTHOR	CASES REPORTED
Allen ⁵	2	Klotz and Simpson ¹⁵	1
Cramer ⁶	1	Lynch and Smith ¹⁶	1
Dreyfus ⁷	3	Maxwell ¹⁷	1
Dible ⁸	2	Middleton ¹⁸	3
Egbert and Geiger ⁹	1	Olson ¹⁹	2
Fine and Jaso ¹⁰	1	Sladden ²⁰	2
Frommel ¹¹	29	Saupe ²¹	2
Gloyne ^{12, 13}	3	Sweaney <i>et al.</i> ²²	1
Harris ¹⁴	4	Stewart and Faulds ²³	1

Some of these authors maintain that carcinoma of the lung in conjunction with pneumoconiosis is comparatively rare. There are those, however, who definitely affirm that inhaled dust is a chronic irritant and as such is a causative factor in the develop-

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ment of the newgrowth. Influenced perhaps by this latter view, the statement is often made in reporting a series of cases of primary pulmonary carcinoma that a given percentage gave a history of dust inhalation. Dust is thereby indirectly, if not directly, assigned an etiological significance.

Before any such generalizations are made, the following points should be proved: (1) that the incidence of pulmonary tumor in individuals with prolonged inhalation of a particular dust is significantly higher than in the general population; and (2) that the dust in question is irritating to the pulmonary parenchyma and is actually capable of producing proliferation and carcinomatous transformation of epithelial tissue.

The purpose of this study is to inquire into these conditions as they are manifested in roentgenological surveys of individuals exposed to industrial dusts; in postmortem observations on cases of pneumoconiosis; and in experimental animals that have inhaled dust over long periods of time.

ROENTGENOLOGICAL SURVEYS

Summary of Literature: The roentgenographical reports on the lungs of 57,362 individuals exposed to dust in various industries throughout the world have been collected from the literature. The

TABLE I

Incidence of Pulmonary Tumors in Clinical and Roentgenological Examinations of Individuals Exposed to Dust, as Reported in the Literature

Number of cases	Non-silicotics	Silicotics	Pulmonary tumor
57,362	45,156	12,206	3 (0.005%)

interpretations as to the presence of nodulation or of neoplasm are presented in Table I. It is to be noted that pulmonary tumor was mentioned in only 3 of the entire group.

Saranac Laboratory Surveys: A study is in progress on individuals exposed to dust in a wide variety of occupations. The results of the surveys thus far completed are given in Table II.

Of the group with nodulation, only 1 individual showed roentgenological evidence of pulmonary tumor. He was 64 years of age and had worked in iron mines for 43 years.

Of the group without nodulation, 2 individuals had developed pulmonary tumor. Their ages were 59 and 68 years respectively. Both had worked in iron mines, the former for 27 years and the latter for 35 years.

In presenting these surveys we realize the difficulties that might attend a roentgenographical diagnosis of pulmonary tumor in silicotic individuals. The same degree of certainty does not obtain as with biopsy or postmortem examination, but lacking these sources of verification it is without question the most accurate method available. For a review of this aspect of the problem reference may be made to the papers of Hirsch ²⁴ and Frothingham.²⁵

TABLE II

Incidence of Pulmonary Tumors as Revealed by Serial Chest Roentgenograms in Individuals Exposed to Dust and Examined in the Saranac Laboratory

Occupation	Number examined	Pulmonary tumor
Iron mines	7,324	3
Foundries	6,613	0
Cement plants	823	0
Gypsum mills and plants	762	0
Copper mines	65	0
Silicotics	1,357	1 (0.074%)
Non-silicotics	14,230	2 (0.014%)
Total	15,587	3 (0.019%)

POSTMORTEM OBSERVATIONS

These observations are of particular significance, for the clinical and roentgenological interpretation of a case may be biased by a history of dust exposure. On examination at autopsy, however, many of these individuals fail to show evidence of pulmonary damage. Some dusts on inhalation into the lung produce no tissue change and are classified as inert. Others are active and cause a marked reaction. Of these, only silica, as Gardner ²⁶ has repeatedly demonstrated, is capable of exciting a characteristic tissue response. This response may be modified by the presence of certain inert substances, such as carbon or iron, or may be complicated by infection, which is usually tuberculous. These differing properties of dust are often neglected by those who maintain that

there is a causal relation between pneumoconiosis and malignancy.

Summary of Literature: The anatomical studies on 444 individuals exposed to various types of dust have been collected. The lungs of these individuals were variously classified as pneumoconiotic, anthracotic, silicotic, anthraco-silicotic, and so on, according to the type of dust inhaled. They have been compiled in Table III.

TABLE III

Malignant Changes Seen at Autopsy in Individuals Exposed to Harmful Dusts, as Reported in the Literature

Number of cases	Malignant changes	Non-pulmonary	Pulmonary	
			Number	Per cent of all autopsies
444	10	4	6	1.3

Important observations from autopsy studies on silicotic individuals have been reported by the Miner's Phthisis Medical Bureau in South Africa.²⁷ The postmortem incidence of primary pulmonary carcinoma in miners dying from all causes during the years 1920-1925 inclusive, has been compiled in Table IV. This inci-

TABLE IV

Primary Carcinoma of the Lung Seen at Autopsy in European Miners and European Males, as Reported by the Miner's Phthisis Medical Bureau of South Africa, 1935

	Total number of autopsies	Carcinoma of lung	Per cent of all autopsies
European miners with silicosis	1438	10	0.70
European miners without silicosis	1679	12	0.71
European males never underground	1393	13	0.93

dence is compared with that in European males never underground and presumably with no history of dust inhalation. The latter is from the statistics of the Johannesburg General Hospital, for the same period.

From these figures it is apparent that pulmonary carcinoma is a rare complication of silicosis in South African gold miners.

Saranac Laboratory Observations: Anatomical studies have

been made on the lungs of 178 males exposed to a variety of industrial dusts for periods of from 1 to 46 years. These studies are presented in condensed form in Table V.

In the entire group there were only 2 males with primary pulmonary carcinoma. One was 59 years of age and had worked in hematite mines for 27 years. The anatomical diagnosis with reference to the pathological changes in the lung caused by the inhaled dust was siderosis without evidence of fibrosis or nodulation. The other individual was 69 years of age and had mined hard coal for 18 years. The pneumoconiosis was interpreted at autopsy as anthraco-silicotic in type.

TABLE V

Incidence of Carcinoma Seen at Autopsy in Individuals Exposed to Dust and Examined in the Saranac Laboratory

Number examined	Years exposed	Silicotics	Carcinoma	
			Pulmonary	Non-pulmonary
178	1-46	136	2 (1.12%)	4

In the remaining 176 cases without pulmonary carcinoma, proliferation of the respiratory epithelial elements was not observed. This obtained even though pulmonary dust fibrosis was often massive and had compressed many epithelial-lined air passages (Figs. 1 and 2).

EXPERIMENTAL OBSERVATIONS

The experiments were designed to elicit the irritative capacity of the dust and its influence on tubercle bacillus infection. Various species of animals were exposed to heavy concentrations of different kinds of dust for long periods of time. The results are compiled in Table VI.

At autopsy, those animals that were exposed long enough showed deposits of dust in the lungs. Where the dust was silicious there was an associated proliferation of connective tissue, which in the case of pure silica resulted in the formation of localized hyaline fibrotic nodules. Often these nodules had formed in lymphoid aggregates contiguous to the mucosa of the major air passages (Fig. 3). In many instances the nodules surrounded and compressed a small epithelial-lined bronchiole. Within the nodules there were

usually one or more small slit-like air spaces lined with low cuboidal epithelium (Fig. 4). Under these circumstances there was every opportunity for irritation to the epithelium by the inhaled dust.

Only 2 animals, both guinea pigs, showed evidence of epithelial proliferation by developing small benign adenomas in the parenchyma of the lung. One guinea pig was exposed to ferruginous chert dust for a period of 749 days; the other had been kept in a soft coal tippie for 390 days.

TABLE VI
*Incidence of Pulmonary Tumors Seen at Autopsy in Animals
that have Inhaled Dust in the Saranac Laboratory*

Type of dust	Guinea pigs	Rabbits	Rats	Chickens	Mice	Cats	Pulmonary tumor
Chalcedony	242	28			40		0
Quartz	894	41	201	12	24	4	0
Marble + quartz	25						0
Hematite + quartz	28						0
Quartz + gypsum	88		27		6		0
Granite	396						0
Chert	196 *	3	52		10		1
Hematite	106		31				0
Asbestos	235						0
Soft coal	37 *						1
Fluorspar — crude			15				0
Carborundum	213						0
Marble	172						0
Gypsum	200		12				0
	2832	72	338	12	80	4	2
Total	3338						0.06%

While none of the inhalation experiments was primarily planned to study the influence of dust on the development of pulmonary carcinoma, one experiment was designed to discover whether or not any strain of a given species was more susceptible than others. For this purpose the reaction to inhaled dust in ordinary albino mice was compared with that in a carcinoma-susceptible strain. There were 40 of the latter exposed to chalcedony over a period of 3 to 12 months. None of these animals manifested an unusual degree of epithelial proliferation in the lungs.

An objection to the interpretation of these experiments might be that strains of animals were employed that were not susceptible

to epithelial proliferation. In 2 guinea pigs, at least, this was not the case, for primary adenomas of the lung were discovered. In the total number of guinea pigs acquired from various sources it seems hardly probable that these should have been the only ones capable of responding by tumor formation when the proper stimulus was applied. If inhaled dust had been such a stimulus it would seem most likely that more of this rather large group should have responded. If, on the other hand, it is proper to perform carcinoma experiments on strains of animals in which spontaneous tumors are infrequent, no one can infer that such tumors have been a common occurrence in this series, since the incidence for the entire group is only 0.06 per cent.

SUMMARY AND CONCLUSIONS

If inhaled dust is of etiological significance in the development of primary pulmonary carcinoma, these two conditions should obtain: the incidence of pulmonary tumor in pneumoconiotic individuals should be higher than in the general population; and the dust in question should be irritating to the pulmonary parenchyma and actually capable of producing proliferation and carcinomatous transformation of epithelial tissue.

A compilation of our observations from roentgenological, post-mortem and experimental studies shows that these conditions do not obtain.

Roentgenological observations, as compiled from the literature on the lungs of 57,362 males exposed to occupational dusts, revealed only 3 cases with primary pulmonary carcinoma, or an incidence of 0.005 per cent. The Saranac Laboratory surveys, which comprise stereoscopic chest roentgenograms of 15,587 males exposed to dust, showed an incidence of 0.019 per cent.

Postmortem examination of 3739 individuals exposed to dust, as compiled from the literature and from our own series, revealed 30 individuals with pulmonary carcinoma, or an incidence of 0.8 per cent. This incidence is lower than that reported in routine autopsy examinations of the general population.

Experimentally, of 3338 animals exposed to many different kinds of dust for long periods of time, only 2 guinea pigs revealed the presence of a pulmonary neoplasm. The tumors in both cases were similar and were interpreted as benign adenomas. All other

animals failed to show evident irritation, hyperplasia or tumor transformation of the epithelium lining the respiratory passages. This was observed irrespective of the activity of the dust, whether it was inert or had caused marked fibrosis of the pulmonary connective tissue.

Inhaled dusts, therefore, except those containing recognized carcinogenic substances such as radium and tar, cannot in general be considered as etiological factors in the development of primary pulmonary carcinoma.

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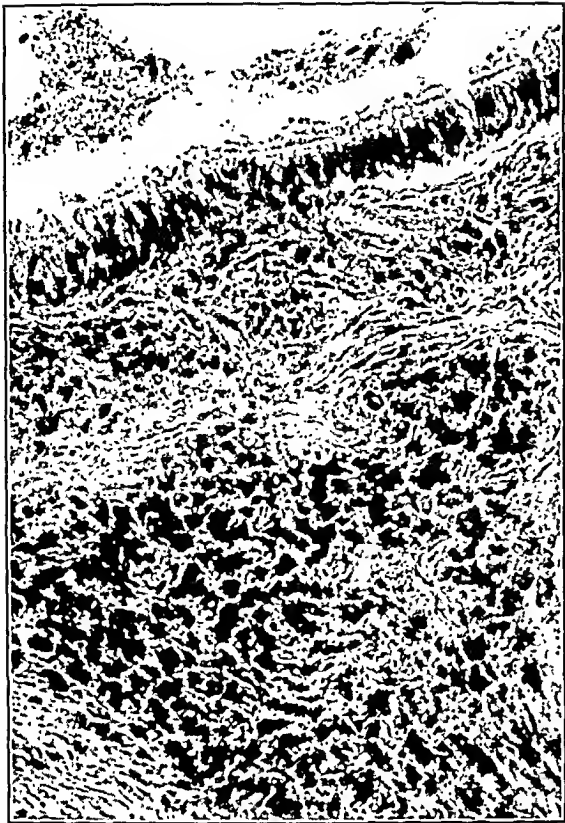
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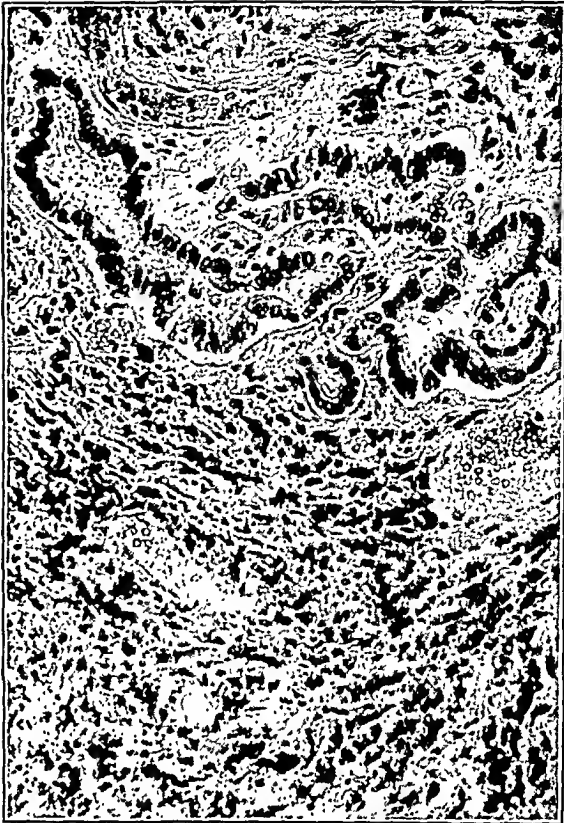
DESCRIPTION OF PLATE

PLATE 15

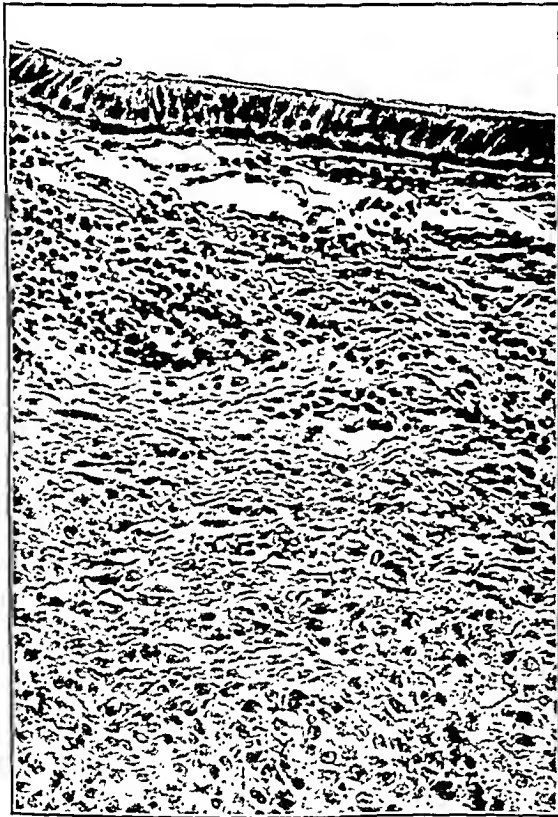
- FIG. 1. Section from the lung of a male, aged 55 years, who had worked in sandstone for 35 years. The section shows the bronchial mucosa covered with a thin layer of dust. There is also marked dust fibrosis in the sub-mucosal connective tissue that is in contact with the basement membrane of the mucosa. Note the absence of epithelial hyperplasia. $\times 210$.
- FIG. 2. Section from the lung of a male, aged 44 years, who had worked in granite for 12 years. The small bronchiole is surrounded and compressed by fibrotic reaction to the inhaled dust. Even under these conditions the epithelium fails to show evidence of irritation or hyperplasia. $\times 210$.
- FIG. 3. Section from the lung of a rabbit that had inhaled quartz dust for 395 days. There is no evidence of irritation to the bronchial epithelium, even though extensive dust fibrosis has occurred in the submucosal connective tissue. $\times 210$.
- FIG. 4. Silicotic reaction in the parenchyma of the lung from the same animal. The air spaces in and about the silicotic nodule are lined with epithelial cells which show no evidence of irritation or hyperplasia. $\times 210$.



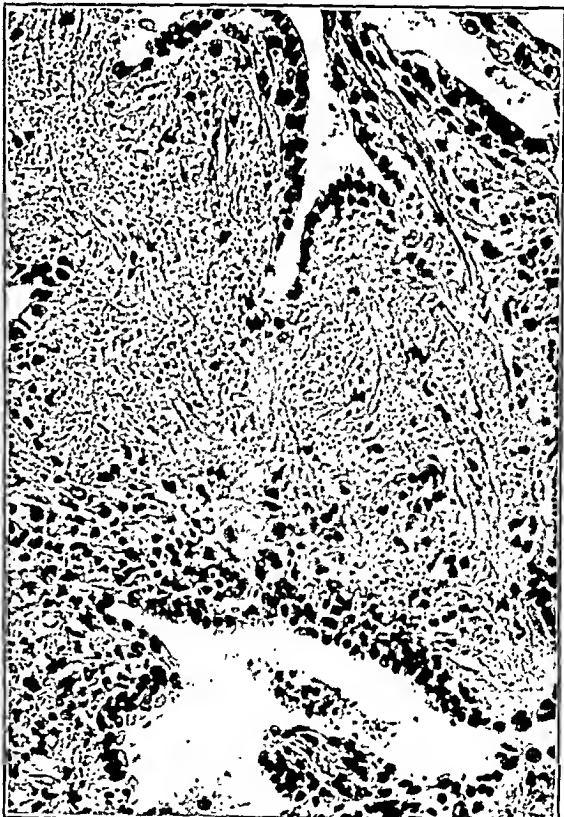
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TUBERCULOUS MENINGITIS AND ITS RELATION TO TUBERCULOUS FOCI IN THE BRAIN *

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In the course of the past 17 years a number of cases of tuberculous meningitis have come under observation in the neuropathological laboratory of the Mount Sinai Hospital. It was noted that in each case the lesion was not limited to the leptomeninges but extended to involve the brain, assuming the character of a meningoencephalitis. In some instances, however, tuberculous lesions were found in the brain substance almost to the entire exclusion of any lesion in the meninges. On several occasions a systematic investigation of the tuberculous lesions and their interrelations was begun, but its completion was deferred until a larger number of cases and better preserved material would be available.

Until very recently it was thought that tuberculous meningitis usually followed the hematogenous dissemination of tubercle bacilli from some focus in the body. This concept was supported by many observations made on brains affected by tuberculosis. When viewed grossly such a brain showed the presence of a massive exudate in the interpeduncular space which tended to spread along the pial vessels in the sulci toward the dorsal surface of the cerebral hemispheres. Histological sections showed considerable alterations in the structure of the blood vessels, consisting of perivascular infiltration and extensive panarteritis with marked narrowing of the lumen. There were also caseation and necrosis.

In 1903 Trevelyan¹ reported that he found among 114 brains affected with tuberculous meningitis 23 that contained tuberculous masses. In addition, he had 10 brains that also contained tuberculous masses but which were not the seat of meningitis. He stated that it was possible for the cerebrospinal fluid to become infected by tubercle bacilli discharged from a focus adjoining the subarachnoid space. In 1924 Kment² examined the chorioid plexus of 27 cases of tuberculous meningitis and found tubercles in 60 per cent. He expressed the opinion that the leptomeningitis was in many

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cases dependent on the formation of tubercles in the chorioid plexus. In 1929, and again in 1933, Rich and McCordock³ reported the results of a study of a large number of cases, on the basis of which they concluded that the concept of the hematogenous route of infection of the cerebrospinal fluid was untenable. They disagreed with Kment's deductions with regard to the tubercles in the chorioid plexus and, in turn, suggested that tuberculous meningitis was due to the rupture of a tubercle adjacent to the subarachnoid space or ventricular cavity, with consequent infection of the cerebrospinal fluid. They traced its origin from such sources in 75 out of 82 cases of tuberculous meningitis. In only 1 case did they consider the chorioid plexus as the source of the meningitis. In 1936 Ragins⁴ reported the results of an investigation of 39 cases of tuberculous meningitis. Contrary to the reports of Rich and McCordock, this author found in 7 cases only indications that older caseous lesions in the brain or meninges had caused the diffuse meningeal infection. From the foregoing it is obvious that the problem of the genesis of tuberculous meningitis is still unsolved and hence the following observations may be considered pertinent.

MATERIAL

The material that formed the subject of this paper consisted of 30 brains. There were 95 additional cases which were not included because the material was not preserved in sufficient quantity to satisfy our demands. All of the 30 cases under consideration were the seat of tuberculous meningitis or tuberculomas. All brains affected with meningitis were cut by the guillotine method into slices about 5 mm. thick. Figure 1 shows the minimum number of sections of each brain that were cut. The brains revealing no tubercles were resectioned into thinner slices 1.5 to 2 mm. thick. As they were cut they were carefully examined for the presence of tubercles or other abnormalities. Sections were taken routinely of the interpeduncular space, the chorioid plexus and the ependyma. In surveying the material histological alterations in the meninges, the cerebral blood vessels, the cerebral substance, the ependyma and the chorioid plexus were examined and the postmortem findings in the other organs were investigated.

ANATOMICAL OBSERVATIONS

Certain of the 30 cases studied exhibited collectively some common pathological features, permitting the arrangement into 5 groups as follows: *Group I*: This consisted of 14 cases showing a diffuse, exudative tuberculous meningo-encephalitis without tubercles in the cerebral substance. *Group II*: This consisted of 3 cases displaying, in addition to the diffuse tuberculous meningo-encephalitis, a few solitary tubercles in the cerebral substance that were not in contact with either the ventricular lining or the sub-arachnoid space. *Group III*: In this division were collected 6 cases, which in addition to the tuberculous meningo-encephalitis present, showed tubercles in the cerebral substance in contact with the leptomeninges. *Group IV*: This was composed of 5 cases which, together with the diffuse tuberculous meningo-encephalitis, presented numerous cortical and subcortical tubercles. *Group V*: This consisted of 2 cases of tuberculoma with the brain free of a diffuse, exudative tuberculous meningitis. The relation of these groups to each other and of the meningeal process to the cerebral lesions formed the basis of this study (Table I).

Group I: In the first group there were 14 cases in which there was a diffuse, exudative meningo-encephalitis of a tuberculous nature. Serial sectioning of the brain did not disclose the presence of tubercles in the cerebral substance. In all of the cases the inflammatory process was not limited to the meninges but tended to extend into the cortex by way of the extensions of the perivascular spaces, resulting in focal areas of secondary encephalitis and encephalomalacia often associated with hemorrhage. In some instances the affected areas displayed slight perivascular accumulations of lymphocytes with congestion of the blood vessels (Fig. 2). In others, there were also foci of necrobiosis (Fig. 3). These lesions, ranging from perivascular accumulations to necrobiosis, can be considered as successive events in one and the same pathological process. In all of the sections the intimate relation of the lesions to the blood vessels indicated that their development was the result of either direct hematogenous dissemination of tubercle bacilli or extension of the meningeal process along the pial vessels. The absence of cortical tubercles in the cases in this group, which formed almost half of the material, offered definite evidence that tuberculous meningitis may develop by other means than the dis-

TABLE I

Analysis of Anatomical Findings in the Brains in 28 Cases of Tuberculous Meningitis and in 2 Cases of Tuberculoma without Meningitis

Serial No.	Post-mortem No.	Age of patient	Presence or absence of tuberculous meningitis	Presence or absence of tubercles or lymphocytic accumulations in the choroid plexus	Presence or absence of tubercles in the cerebral substance. If present, location or number of tubercles is stated	Presence or absence of tubercles in contact with the leptomeninges
<i>Group I</i>						
1	9685	5 yrs.	Present	Normal plexus	Absent	Absent
2	9819	19 "	Present	Normal plexus	Absent	Absent
3	9326	32 "	Present	Normal plexus	Absent	Absent
4	D3	—	Present	Normal plexus	Absent	Absent
5	6674	3 yrs.	Present	Tubercle	Absent	Absent
6	10286	3 "	Present	Tubercle	Absent	Absent
7	9684	3 "	Present	Tubercle	Absent	Absent
8	9382	20 "	Present	Tubercle	Absent	Absent
9	9249	21 "	Present	Tubercle	Absent	Absent
10	9231	26 "	Present	Tubercle	Absent	Absent
11	9097	33 "	Present	Tubercle	Absent	Absent
12	9104	1½ "	Present	Lymphocytic accumulation	Absent	Absent
13	10060	27 "	Present	Lymphocytic accumulation	Absent	Absent
14	10276	52 "	Present	Lymphocytic accumulation	Absent	Absent
<i>Group II</i>						
15	9537	5½ yrs.	Present	Lymphocytic accumulation	Basis pontis and left frontal lobe	Absent
16	8147	24 "	Present	Normal plexus	Rt. frontal lobe	Absent
17	9540	1¾ "	Present	Tubercle	Brachium pontis and left frontal lobe	Absent

<i>Group III</i>						
18	9296	16 mos.	Present	Tubercle	Island of Reil	Present
19	7799	5 yrs.	Present	Lymphocytic accumulation	Rt. occipital lobe	Present
20	6762	19 "	Present	Lymphocytic accumulation	Cerebellum	Present
21	10173	43 "	Present	Normal plexus	Cerebrum	Present
22	6808	9 "	Present	Tubercle	Rt. occipital lobe	Present
23	8217	9 "	Present	Lymphocytic accumulation	Cerebellum	Present
<i>Group IV</i>						
24	9933	15 mos.	Present	Normal plexus	18 tubercles	Present
25	5026	2 yrs.	Present	Normal plexus	56 tubercles	Present
26	9378	5 "	Present	Tubercle	10 tubercles	Present
27	6993	8 "	Present	Section of plexus lost	110 tubercles	Present
28	9638	42 "	Present	Lymphocytic accumulation	30 tubercles	Present
<i>Group V</i>						
29	7838	36 yrs.	Slight	Tubercle	Frontoparietal and	Present
30	9381	50 "	Absent	Normal plexus	Rt. occipital lobe	Present
					Cerebrum	

charge of bacilli from a cortical lesion. The pathological changes described in the cerebral substance appeared to be secondary to the meningeal process or else a coincidental development.

Group II: This group consisted of 3 cases in which, along with the diffuse tuberculous meningo-encephalitis, tubercles were found that were not in contact with either the ventricular lining or the pia mater. These cases differed from those in the first group only by the presence of these solitary tubercles. In Case 9537 there was one tubercle at the basis pontis and another in the gray matter of the left occipital lobe. In Case 8147 there was a tubercle in the white matter of the right frontal lobe. In Case 9540 there was one tubercle in the brachium pontis and another in the left frontal lobe, at the junction of the white and gray matter (Fig. 4). The remote location of these tubercles makes it improbable that they were the source of the infection of the subarachnoid space. Hence it is permissible to conclude that the meningitis in these cases was not the result of the presence of these tubercles.

Group III: This group contained 6 cases in which, in addition to the tuberculous meningo-encephalitis, there were found tubercles in contact with the ependyma or leptomeninges. It will be recalled that such lesions were regarded by Rich and McCordock³ as the foci from which tubercle bacilli entered the cerebrospinal fluid. It would seem that most of these lesions, in themselves, do not offer any evidence either for or against such a hypothesis. However, since in the entire series these were the only cases that might fit in with such a theory, they will be described in detail. In Case 9296 there was a tubercle 4 mm. in diameter in the gray matter at the Island of Reil. The caseous process extended directly into the adjacent sulcus. Figure 5 which was taken from this lesion shows, in addition to the tubercle, two smaller areas of infiltration in the cortical tissue. It is not possible by any positive means to determine whether such a lesion was the result or the cause of the meningeal process, or whether it developed simultaneously with the latter. In Case 7799 there was a tubercle 3 mm. in diameter at the base of a sulcus in the right occipital lobe. The meningeal reaction at the site of the lesion was minimal (Fig. 6). In this case, also, one cannot determine from the available evidence which was the primary lesion, the tubercle or the meningitis. In Case 6762 there was a conglomerate tuberculous focus in the cerebellum

occupying an area 1 cm. in diameter. The inflammatory process extended into the neighboring sulci (Fig. 7). In Case 10173 there were a few instances of caseous tubercles in contact with the leptomeninges (Fig. 8). In Case 6808 there was a large tubercle (tuberculoma), 5 by 3 cm., which was situated in the right occipital lobe occupying the space between the descending horn of the lateral ventricle and the ventral aspect of the lobe. The last case, 8217, presented a large tuberculoma measuring 1.5 by 3.5 cm. On the opposite side there were two adjacent tuberculomas, together measuring 2.5 by 1 cm.

Group IV: This group consisted of 5 cases, characterized by the presence of numerous tubercles throughout the brain (Fig. 9). The brain findings in these cases, in the multiplicity of the lesions, resembled closely the lungs, spleen and kidneys in instances of generalized miliary or disseminated tuberculosis. It may reasonably be assumed that these lesions were the result of direct hematogenous dissemination and thus a correlation between the distribution of such tubercles and that of the vascular supply to the brain may be of interest. There was a total of 224 tubercles in the 5 brains in this group. The right and left side of each brain were affected to nearly the same extent. When classified in relation to the particular artery involved, it was found that 51.8 per cent of the tubercles were in the area vascularized by the middle cerebral artery, 33 per cent in the zone supplied by the anterior cerebral artery, and 15.2 per cent in parts supplied by the posterior cerebral artery. The cortical system of arteries accounted for the vast majority of the lesions, as compared to the very small number of lesions in zones vascularized by the central system. This is in keeping with the fact that the cortical system nourishes a volume of cerebral tissue far in excess of that supplied by the latter. When the tubercles were tabulated according to whether they were situated in the cortical or subcortical areas of the brain, it was found that the former contained 40 per cent of the lesions, the latter 38 per cent, while 22 per cent were situated at the junction of the cortex and the subcortex. The tubercles varied in size from 1 to 3 mm., although in some instances many were so close together as to form fairly large conglomerate masses. To cause such generalized dissemination large numbers of tubercle bacilli must have been circulating in the blood stream, and it is likely that the meningeal

infection, present in all the cases in this group, was due to the hematogenous dissemination, developing at the same time as the tubercles in the cerebral substance.

Group V: This group includes 2 cases of tuberculoma without meningitis, which indicates that tuberculous meningitis need not follow the presence of a tuberculous focus adjoining the ventricular cavity or subarachnoid space. The first, Case 7858, was that of a female, aged 36 years, who was subjected to an exploratory craniotomy for a neoplasm in the left cerebral hemisphere. Six days afterward she developed an elevation of temperature and the spinal fluid, which was previously normal, contained 656 cells per cmm. She died 2 weeks later. The brain disclosed 2 tuberculomas. One was 2 cm. in diameter and was situated in the left parieto-temporal region, adjacent to the descending horn of the lateral ventricle. The ependymal lining between the tuberculoma and the ventricle showed evidence of disintegration without actual loss of continuity. The second lesion was only 5 mm. in diameter and was situated at the base of a sulcus in the right occipital lobe (Fig. 10). The pia arachnoid membrane was not thickened at the site of this lesion although there was an exudate in the adjacent sulcus. The chorioid plexus showed early tubercle formation. The brain displayed no diffuse exudative tuberculous meningitis, despite the fact that in some sections there were a few large and small round cells in the subarachnoid space. Case 9381 was that of a 50 year old male negro who was operated upon on two occasions for an expanding intracranial lesion. The brain, when sectioned, revealed the presence of 3 tuberculous lesions. One was situated in the cortex of the brain and measured 4 by 3.5 cm. It was in direct contact with the subarachnoid space. The meninges were thickened and adherent to its surface. In the right cerebral hemisphere there was a second lesion measuring 3 by 4.5 cm. The inflammatory process extended into the neighboring sulcus but there was no meningeal reaction (Fig. 11). In the right occipital lobe there was a third lesion measuring 0.5 cm. in diameter. There was no exudate at the base of the brain. Miliary tubercles were found in the lungs, liver, spleen and kidneys. The cases in this group are instances in which caseous lesions were situated in direct contact with the leptomeninges and yet, diffuse exudative tuberculous meningitis was absent.

THE CHORIOID PLEXUS

In the series studied there were 11 cases in which tubercle formations were found in the chorioid plexus. In an additional 8 cases histological examination revealed accumulations of cells of the small and large round cell type. All of the tubercles were microscopic in size. Although they were in direct contact with the circulating cerebrospinal fluid, thus enhancing the probabilities of the latter becoming infected by such tubercle bacilli as might be discharged, yet the extremely small size of the tubercles leads one to question whether or not they could discharge sufficient numbers of bacilli to cause the widespread, exudative reaction usually seen at the base of the brain. At best, it can be said that the tubercles and cellular accumulations are indicative of the presence of bacilli circulating in the blood stream.*

TUBERCULOUS MENINGITIS AFFECTING THE SPINAL CORD

In 3 of the cases studied the subarachnoid space around the spinal cord was the seat of a tuberculous exudate as marked in degree as that found at the base of the related brain. Sections of the spinal cord revealed areas in the nerve trunks that were the seat of inflammatory reaction, though they were not in direct anatomical continuity with the subarachnoid space. There was a marked lymphocytic infiltration in the connective tissue between the nerve bundles and among the nerve fibers (Fig. 12). The infiltration was most dense around the blood vessels. In 1 case there were giant cells and tubercle formation in the nerve roots. While the infection here could readily extend to the pia arachnoid space enveloping the nerve roots, its ultimate spread to the endoneurium and perineurium was most likely hematogenous in origin, and due to extension by means of the small blood vessels which ramify in the connective tissue surrounding the nerve bundles.

RELATION OF TUBERCULOUS MENINGITIS TO TUBERCULOUS INFECTION IN OTHER ORGANS OF THE BODY

One of the arguments that has been advanced against the hematogenous theory is that tuberculous meningitis does not occur in

* A finding which apparently has no bearing on this problem but which may be mentioned in passing is that in 53 per cent of the cases psammoma bodies were found in the chorioid plexus.

all cases of miliary tuberculosis; also, that cases of meningitis may take place without a generalized miliary tuberculosis. One hundred cases of tuberculous meningitis in which there were complete postmortem examinations were surveyed. A comparison of the findings in the organs of the body with those in the brain was made. It was noted that the spleen was affected in 76 per cent of the cases, the lungs in 69 per cent, the liver in 64 per cent and the kidneys in 50 per cent. These figures differ to a slight extent from those published by other authors (Paterson,⁵ MacGregor, Kirkpatrick and Craig,⁶ and Blacklock and Griffin⁷). In this series the spleen was affected in a greater percentage of cases than is usually reported. Organs such as the pancreas, thymus, adrenals and thyroid, were affected in only a small percentage of cases. The meninges apparently belong in the same category as the organs just mentioned. Thus, for reasons not understood, tuberculous meningitis need not develop in all cases of disseminated and miliary tuberculous disease.

Rich and McCordock have concluded that other serous cavities such as the pleural, pericardial and peritoneal, behave with regard to tubercles in a manner similar to that which they postulate for the meningeal space, claiming that, in such cavities, rupture of a tubercle adjacent to the cavity discharges its contents, resulting in an exudative inflammation. This is out of accord with the findings recorded in the postmortem protocols of 100 completely autopsied cases of tuberculous meningitis which were surveyed by us. In this series miliary tubercles on the pleural lining were found in 12 per cent, on the pericardial lining in 7 per cent, and on the peritoneum in 10 per cent. In nearly all of the cases the cavities were normal. In a few instances the peritoneum showed localized, caseous fibroblastic thickening surrounding tuberculous ulcers of the intestines. This again leads one to conclude that the presence of a tubercle in the wall of a serous cavity does not necessarily result in a tuberculous infection of that cavity.

COMMENT AND SUMMARY

The anatomical survey of 28 cases of tuberculous meningo-encephalitis and of 2 cases of tuberculoma without meningitis resulted in the following observations:

In a large proportion (14 cases) there was a diffuse, exudative tuberculous meningo-encephalitis without the presence of tubercles

in the cerebral substance. As part of the inflammatory process there were, in the cortex, small foci of encephalorrhagia, perivascular infiltration, lymphocytic accumulations and necrobiosis. These changes were in relation to the blood vessels.

A small proportion (3 cases) showed, in addition to the diffuse tuberculous meningo-encephalitis, a few solitary tubercles in the cerebral substance which were not in contact with either the ventricular lining or the leptomeninges. It is unlikely that they could have served as foci from which the meningitis developed.

In a small quota (6 cases) there were, in addition to the tuberculous meningo-encephalitic process, tuberculous lesions in the cerebral substance, which were in contact with the leptomeninges. The lesions in 4 of these cases were cortical tubercles. The remaining 2 cases in this group revealed large tuberculomas giving clinical manifestations of a cerebral neoplasm.

In another group (5 cases) there were numerous solitary cortical and subcortical tubercles in the cerebral substance. These were present along with the diffuse, tuberculous meningo-encephalitis. The great number of tubercles suggests that they were the result of direct blood stream dissemination.

In 2 cases tuberculomas in contact with the ventricular lining or with the leptomeninges were present without a concurrent, diffuse exudative meningitis.

The evidence presented shows that in all cases of tuberculous meningitis there was an extension of the inflammatory process into the cortex resulting in foci of encephalitis, which varied in degree from perivascular infiltration to tubercle formation. In a few cases solitary tubercles were found but these were not in contact with the ependyma or leptomeninges. When tubercles occurred in great numbers they were due to direct hematogenous dissemination. Large solitary tuberculomas occurred with or without meningitis. In only 6 cases in this series were cortical tubercles demonstrated which might have been responsible for the coexistent meningitis. In 11 cases the chorioid plexus disclosed the presence of tubercle formation. The blood vessel changes which were observed during the course of study revealed no findings that differed from those already described by other observers.

NOTE: Our appreciation is due to Dr. Joseph H. Globus, under whose supervision the work was performed, for his advice and helpful criticism during the course of this work.

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DESCRIPTION OF PLATES

PLATE 16

FIG. 1. Photographs illustrating the minimum number of sections cut from each brain.



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Tuberculous M.

PLATE 17

- FIG. 2. Case 10060. Section showing tuberculous exudate in subarachnoid space with perivascular foci of lymphocytes in cortex. H & E stain.
- FIG. 3. Case 10060. Section showing exudate in subarachnoid space with cortical focus of necrobiosis and lymphocytic accumulation. H & E stain.
- FIG. 4. Case 9540. Section showing tubercle at the junction of white and gray matter, remote from leptomeninges. H & E stain.
- FIG. 5. Case 9296. Section showing tuberculous focus at the base of a sulcus. H & E stain.
- FIG. 6. Case 7799. Section showing tuberculous focus at the base of a sulcus with area of encephalomalacia. Note minimal meningeal reaction. H & E stain.



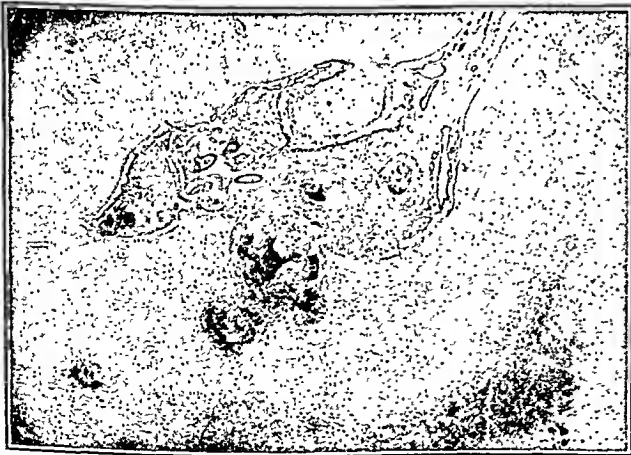
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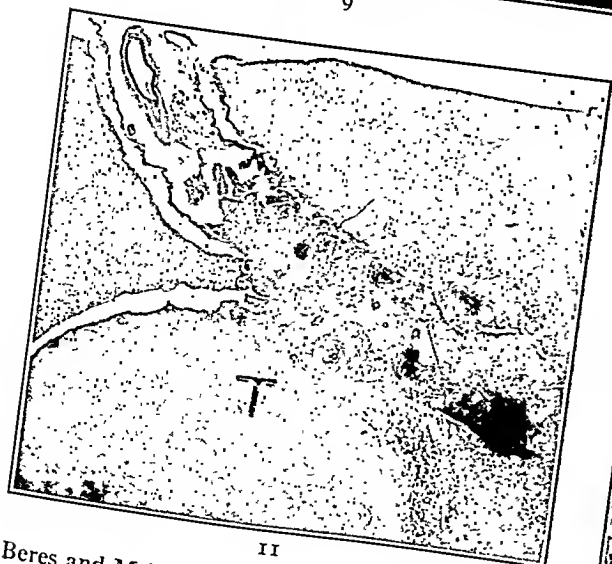
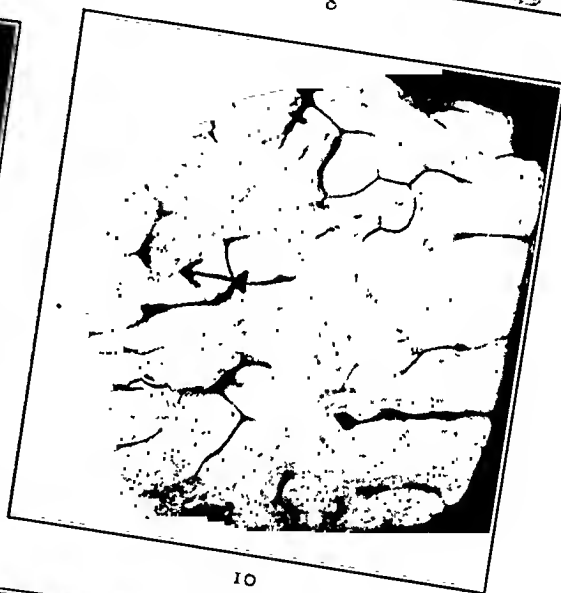
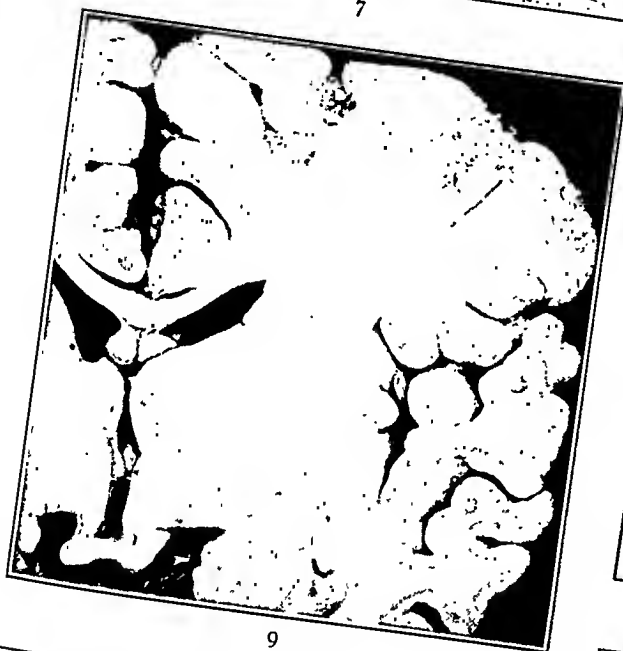
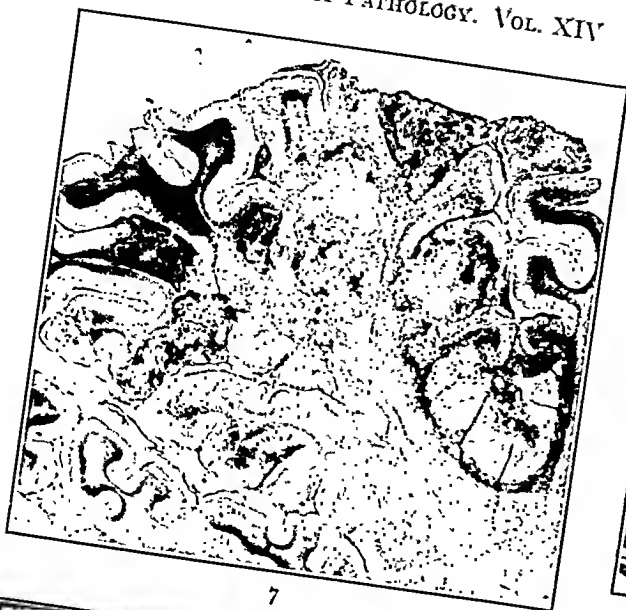
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PLATE 18

- FIG. 7. Case 6762. Section showing conglomerate tuberculous focus in cerebellum. H & E stain.
- FIG. 8. Case 10173. Section showing tubercle in contact with leptomeninges. H & E stain.
- FIG. 9. Case 6993. Photograph illustrating numerous solitary tubercles in cerebral hemisphere. There were 110 tubercles in this brain.
- FIG. 10. Case 7858. Photograph illustrating tubercle adjacent to a sulcus. No diffuse exudative meningitis was present.
- FIG. 11. Case 9381. Section showing tuberculoma (T) in contact with leptomeninges. No diffuse exudative meningitis was present. H & E stain.
- FIG. 12. Case 9685. Section of spinal nerve trunk showing a giant cell (arrow) and lymphocytic infiltration into the connective tissue. H & E stain.



Beres and Meltzer

Tuberculous Meningitis

AN EXPERIMENTAL STUDY OF COMPLEMENT AND HEMOLYTIC AMBOCEPTOR INTRODUCED INTO CHICK EMBRYOS *

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Recent investigations in this laboratory and elsewhere have shown that a number of viruses will infect the tissues of embryo chicks.^{1, 2} We have utilized the same technique to study bacterial infection, demonstrating that many microorganisms pathogenic for man will likewise infect the chick embryo and cause pathological lesions in several respects quite similar to those observed in the human host.

It is a striking fact that as yet no convenient experimental hosts have been found in which infectious lesions, analogous to those encountered in many specific diseases of the human being, can be induced with pure cultures of the respective bacterial incitants. This applies, for example, to such familiar diseases as typhoid fever, diphtheria, epidemic meningitis, gonorrhea, soft chancre, *H. influenzae* infections, whooping cough and others.

In order that studies of the pathogenesis and immunology of such infections may be facilitated it is desirable that experimental hosts be utilized in which lesions comparable with those observed in the respective human diseases may be readily and conveniently reproduced.

Preliminary studies in this laboratory have indicated that the chick embryo can be utilized for investigations of this character, and more extensive research might demonstrate that this sterile living host has a very broad applicability to the study of such problems.

For example, Goodpasture and Anderson have shown that the chick embryo can be infected with pure cultures of such bacteria as staphylococcus, streptococcus, *E. typhi*, *C. diphtheriae*, *Br. abortus*, among others; and that some lesions simulate those encountered in the natural hosts.³ More recently Buddingh and Polk⁴

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have reported that experimental infection with meningococci simulates that in man, and Gallavan,⁵ and Gallavan and Goodpasture⁶ have described lesions in embryos infected with *H. influenzae* and *H. pertussis* which correspond quite closely to those occurring in man.

Because of the evident value of the chick embryo technique for the study of infectious diseases, it has appeared quite likely that this host might advantageously be used also for investigations concerning phenomena of susceptibility, resistance and immunity.

There are certain obvious advantages that the chick embryo offers for the study of infection. It is, of course, an organized living host whose tissues are sterile. It is a relatively uniform medium because it has not yet been subjected to infective disease and consequent immunity reactions, or to dietary changes, maturation and other incidents of independent life in the exterior environment. It is of course an accessible, cheap and convenient experimental host.

Because chick embryos have been found to be susceptible to infection by a number of human pathogens, it seems evident that they might be utilized for investigations not only of natural protective responses, but as passive vehicles to evaluate the significance of experimentally introduced antibodies in the serums of immune animals and other substances designed to influence the course of infection. The proved protective effect of diphtheria antitoxin introduced into the embryo against infection with *C. diphtheriae* is an illustration.³

In an attempt to develop a satisfactory method for these purposes it seemed advisable to investigate such matters as the presence in the chick embryo of native agents that are considered to play a part in combating infection, for example complement, and the fate of artificially introduced complement and antibodies.

In the first place we tested embryonic material for the presence of complement, using an antishoop amboceptor system. No native complement being demonstrated with this system, we introduced guinea pig complement and followed its distribution and fate. We then introduced antishoop amboceptor by various routes, because of its relative stability and ease of demonstration, and followed its distribution in the fluids of the host. Finally complement and amboceptor were introduced into embryos to circulate in the blood concurrently.

By these experiments we wished to determine among other things the practicability of introducing similar foreign substances into the embryo in quantities adequate to the study of their effects in later investigations of active and passive resistance of the embryo to specific infectious agents.

Natural Complement in Chick Embryos

Rywosch ⁷ found no lysin for rabbit erythrocytes in the serum of chick embryos before the 21st day. She failed likewise to demonstrate complement. She stated, however, that bacteriolysins for *E. coli* were present in extremely small amounts from the 14th day onward. Sherman ⁸ found that lysins for rabbit erythrocytes did not appear until the 21st day of incubation, when the chick was pecking through the shell. Complement for rabbit but not for sheep erythrocytes was found in small amounts in embryos on the 17th day of incubation.

For our work we chose first to test the presence of complement for sensitized sheep cells in serum, cavity fluids and tissues of the embryo, and in the serum of recently hatched chicks and young and adult chickens.

Technique for Collecting Serum and Fluids

Adult and half-grown chickens were bled from the wing vein with a needle and syringe. Blood of newly hatched and embryonic chicks was aspirated directly from the exposed heart. Chorion-allantoic and amniotic fluids were collected by puncturing the respective membranes with capillary pipettes and aspirating the fluids. Tissue extracts were prepared by pooling the heart, lung, spleen, liver and kidneys of several embryos. These tissues were ground in a mortar and diluted with twice their volume of 0.85 per cent saline. All the materials to be tested were centrifuged to obtain a clear supernatant fluid.

Test for Complement: Sheep cells sensitized with rabbit anti-sheep hemolysin were used to test for complement. The reagents were set up in the following proportions:

Antisheep hemolysin diluted to contain 1 unit in	0.1 cc.
Washed red cells (sheep), 2 per cent	0.1 cc.
Salt solution, 0.85 per cent	0.3 cc.
Fluid to be tested for complement	0.1 cc.

With each experiment a positive control, using pooled fresh guinea pig serum for complement, was run as well as a negative salt solution control.

The tests were always performed within 1 to 2 hours after the fluids were collected. Typical results are recorded in Table I.

TABLE I
Complement Content of Chicken Serum

Serum dilutions	Guinea pig serum (control)	Serums of (6) adult chickens	Serums of (3) half-grown chickens	Serums of (3) 2-day-old chickens
No amboceptor (control)	—	—	—	—
Dilution 1 : 30	+++	—	—	—
Dilution 1 : 5	+++	+++	++	—
Dilution 1 : 0	+++	+++		++

+++ = complete hemolysis.
— = no hemolysis.

No complement whatever was demonstrated in undiluted serum, cavity fluids and tissue extracts of 17, 19 and 20-day embryos. Two-day-old chicks contain a small amount of complement. It is increased in amount in half-grown chicks and is quite abundant in adult fowls. This is in agreement with previous work.

Recovery of Guinea Pig Complement after Introduction into Chick Embryos

Complement Dropped onto the Chorio-Allantoic Membrane: On 3 successive days 0.4 cc., 0.1 cc., and 0.1 cc. respective amounts of fresh guinea pig serum complement were dropped onto the chorio-allantoic membrane of chick embryos 16 days old, exposed by the coverslip method.⁹ Twenty-four hours after the last dose the embryos were killed and tested for complement. None could be demonstrated in the blood serum or organ extract, but it was present in the chorio-allantoic fluid in a concentration so that 0.1 cc. was sufficient to hemolyze completely one portion of sensitized sheep red corpuscles.

Complement Injected into the Body of the Embryo: Embryos of 16 days incubation received into their bodies by injection 2 cc. of fresh guinea pig serum. After 24 and 72 hours they were tested, but no complement was found in the blood serum or organ ex-

tracts. However, partial hemolysis of one portion of sensitized red sheep cells was produced by the chorio-allantoic and amniotic fluids 72 hours after the injection.

Complement Injected Intravenously: Injection of 0.2 cc. amounts of fresh guinea pig serum was made intravenously (tech-

TABLE II
Demonstration of Complement after Intravenous Injection

Time after injection, (x) embryo each	Blood serum	Chorio-allantoic fluid	Amniotic fluid	Organ extract	Control complement *
5 min.	+++	--	--		+++
2 hrs.	++	--	--	+	+++
4 hrs.	++	--	--		+++
16 hrs.	+	++	--	+	+++
24 hrs.	-	++	--		+++

* The control complement was diluted 1:10 and kept at 37° C.

nique follows) into embryos of 12 days incubation. After varying intervals the embryonic fluids were tested for complement. The results are recorded in Table II.

These experiments indicate that guinea pig complement in the quantities used disappears from the circulation in about 16 hours. Complement placed on the chorio-allantoic membrane or injected directly into the body of the embryo, if absorbed, does not persist in demonstrable quantity in the blood serum after 24 hours. Although leakage into the chorio-allantoic fluid was not certainly eliminated there is a possibility that some complement was excreted into this fluid. Guinea pig complement seems to be fairly rapidly inactivated *in vivo* in these experiments.

Intravenous injection is the method of choice for assuring the presence of complement in the circulating blood for a few hours. After 16 hours it had almost disappeared from the serum, although it was still demonstrable after 24 hours in the chorio-allantoic fluid, into which it was probably excreted.

The degree of dilution of complement within the internal and external fluids of the embryo in these experiments is not known. The average egg used weighed about 60 gm. The total volume of the embryonic tissue fluids, and especially the total blood volume,

is relatively small, and it is evident from the experiments that the dilution in serum, at least for several hours, is within a range that permits complement to remain effective in the tests used.

Recovery of Antisheep Hemolysin after its Introduction into the Incubating Egg

Normal chick embryo serum contains no hemolytic amboceptor for sheep cells. Rabbit hemolysin having a hemolytic titer for

TABLE III

Recovery of Hemolysin after Dropping it onto the Chorio-Allantoic Membrane

Material tested	Embryo blood serum	Chorio-allantoic fluid	Amniotic fluid	Organ extract
<i>Group 1.</i> Pooled fluids of 10 embryos 24 hrs. after dropping 0.2 cc. of hemolysin in 0.8 cc. of saline	++	+++		—
<i>Group 2.</i> Pooled fluids of 6 embryos 24 hrs. after dropping 0.1 cc. of hemolysin in 0.9 cc. of saline	—	+++	—	—
<i>Group 3.</i> Pooled fluids of 3 embryos 72 hrs. after dropping 0.1 cc. of hemolysin in 0.9 cc. of saline	—	+++	—	—
<i>Group 4.</i> Pooled fluids of 7 embryos 24 hrs. after dropping 0.2 cc. of hemolysin in 0.8 cc. of saline	—	+++	+++	—
<i>Group 5.</i> Pooled fluids of 5 embryos 24 hrs. after dropping 0.1 cc. of hemolysin in 0.4 cc. of saline	—	+++	+++	—

+++ = complete hemolysis.

++ = partial hemolysis.

+ = slight hemolysis.

— = no hemolysis.

sheep cells not less than 1:1000 was used in these experiments. It was introduced into the chick embryo by the various methods described below and its presence or absence in the fluids and tissues was determined by testing their hemolytic power for sheep cells in the presence of fresh guinea pig complement. The materials to

be tested were collected and prepared by the same methods as those described under the complement determination. The materials for the test were mixed in the following proportions:

Fluid to be tested for hemolysin	0.1 cc.
Salt solution, 0.85 per cent	0.3 cc.
Washed red corpuscles (sheep)	0.1 cc.
Fresh guinea pig complement (0.1 cc. solution hemolyzes 0.1 cc. sensitized cells)	0.1 cc.

TABLE IV

Tests for Hemolysin after Injection into Yolk Sac

Material tested	Chick embryo blood serum	Chorio-allantoic fluid	Amniotic fluid	Organ extract
<i>Group 1.</i> Pooled fluids of (4) embryos 24 hrs. after injection of 0.2 cc. of hemolysin in 0.8 cc. of saline	±	+++	+++	—
<i>Group 2.</i> Pooled fluids of (4) embryos 48 hrs. after injection of 0.1 cc. of hemolysin	—	—	—	—
<i>Group 3.</i> Pooled fluids of (4) embryos 96 hrs. after injection of 0.1 cc. of hemolysin	—			—

Hemolysin Dropped onto the Chorio-Allantoic Membrane

Using the coverslip technique measured amounts of hemolysin were dropped onto the exposed chorio-allantoic membrane. The embryos were returned to the incubator at 37° C. and examined at varying intervals. The results are recorded in Table III.

Recovery of Hemolysin after Injection into Yolk Sac

The yolk sac of eggs containing 16 or 17-day-old chick embryos received by injection measured amounts of hemolysin, and tests for its presence were made after various intervals. The results are recorded in Table IV.

Recovery of Hemolysin after Injection into Albumin Sac at an Early Stage of Development

In this experiment a slit was cut in the small end of eggs containing 2, 3, 4 and 6-day-old embryos. Through the slit measured

amounts of hemolysin were injected into the albumin sac which lies at this end. The injection was made with needle and syringe. The holes were then sealed with paraffin and the eggs kept in the incubator until the 14th day of incubation when they were examined for hemolysin. The results are tabulated in Table V.

TABLE V
Tests for Hemolysin after Injection into Albumin Sac

Material tested	Blood serum *	Chorio-allantoic fluid	Amniotic fluid
<i>Group 1.</i> 2-day-old embryos injected with 0.2 cc. hemolysin	(1) —	—	+++
	(2) ±	—	+++
<i>Group 2.</i> 3-day-old embryos injected with 0.2 cc. hemolysin	(1) ±	±	+++
	(2) —	±	+++
	(3) ±	±	+++
<i>Group 3.</i> 4-day-old embryos injected with 0.2 cc. hemolysin	(1) —	±	±
	(2) —	—	±
<i>Group 4.</i> 6-day-old embryos injected with 0.2 cc. hemolysin	(1) —	—	—
	(2) —	±	+

* Each number refers to a different embryo.

The concentration of the hemolysin in the amniotic fluid is due probably to the opening of the sero-amniotic junction that occurs in the chick embryo about the 12th day when the proteins from the albumin are liberated into the amniotic fluid.

Recovery of Hemolysin after Injection into the Body of the Embryo

Fourteen to 16-day-old embryos were exposed by the coverslip technique. The chorio-allantoic and amniotic membranes were pierced and the hemolysin injected directly into the embryo by means of a needle and syringe. It was not always possible to be sure what part of the embryo received the hemolysin.

These results show that the hemolysin is absorbed into the blood stream from the body in most cases, but not all. In an effort to learn why the material introduced in this manner cannot always be recovered from the blood stream a number of injections of methylene blue were made in chick embryos from which the shells

had been completely removed so that the whole chick could be observed. The methylene blue could be seen through the thin skin of the embryo. It diffused through the tissues but could often be seen flowing back out of the hole made by the injecting

TABLE VI
Tests for Hemolysin after Injection into Bodies of Embryos

Material tested	Blood serum	Chorio-allantoic and amniotic fluid	Organ extract
<i>Group 1.</i> (6) 16-day-old embryos 24 hrs. after injection	+++	+++	+
<i>Group 2.</i> (3) 16-day-old embryos 48 hrs. after injection	++		±
<i>Group 3.</i> (6) 14-day-old embryos 92 hrs. after injection	+++	+++	—
<i>Group 4.</i> (7) 16-day-old embryos 96 hrs. after injection	+++		
<i>Group 5.</i> (8) 14-day-old embryos 24 hrs. after injection	±	+++	—
<i>Group 6.</i> (6) 14-day-old embryos 48 hrs. after injection	+	++	—
<i>Group 7.</i> (6) 14-day-old embryos 24 hrs. after injection	+++	+++	—
<i>Group 8.</i> (6) 14-day-old embryos 24 hrs. after injection	±	+++	
<i>Group 9.</i> (8) 14-day-old embryos 24 hrs. after injection	+++	+++	
<i>Group 10.</i> (5) 14-day-old embryos 24 hrs. after injection	+++	+++	
<i>Group 11.</i> (3) 14-day-old embryos 48 hrs. after injection	+++	+++	
<i>Group 12.</i> (4) 14-day-old embryos 72 hrs. after injection	—	+++	
<i>Group 13.</i> (4) 14-day-old embryos 72 hrs. after injection	++	+++	

needle. In one instance, after a supposedly intraperitoneal injection, the methylene blue flowed out of the mouth of the chick. A number of uncontrolled factors determine whether the injected hemolysin remains to be absorbed or flows out into the surrounding fluids.

Recovery of Hemolysin after Intraperitoneal Injection

One attempt to control the point of injection was made. The embryos were exposed by removing about a square inch of shell. Through this a small hole was made in the membranes and the leg of the chick was pulled through with sterile forceps. The injection was made by passing the needle through the firm part of the thigh into the peritoneum. The firmer tissues of the thigh kept the material from flowing out and the hemolysin was recovered from the

TABLE VII

Recovery of Hemolysin after Injection through Leg into Peritoneum

Material tested	Blood serum	Chorio-allantoic and amniotic fluid
<i>Embryo 1.</i> 15 days old, 24 hrs. after injection	+++	—
<i>Embryo 2.</i> 15 days old, 24 hrs. after injection	+++	++
<i>Embryo 3.</i> 15 days old, 24 hrs. after injection	++	—
<i>Embryo 4.</i> 15 days old, 24 hrs. after injection	+++	+
<i>Embryo 5.</i> 15 days old, 24 hrs. after injection	+++	+

blood stream in each instance where the embryo was 15 or more days old. But in the younger chicks the legs are too small and too soft to make the injection practicable. Another disadvantage of this method lies in the fact that the membranes were always considerably torn by pulling the leg through them.

Recovery of Hemolysin after Intravenous Injection

To make this injection the large vessels of the membrane must be accurately located by candling the eggs under a strong light. The membranal vessels may be distinguished from the deeper ones by the fact that the former are fixed in the membrane whereas the latter may be seen moving freely in the embryo fluids. An area about half an inch square is marked on the shell to indicate the position of the largest membranal vessel. At this point the eggshell is cut and opened by the coverslip technique. A tuberculin syringe with a 27 gauge needle is used for the injection. The direction of

the blood flow must be determined by slipping the needle under the vessel until the lumen is occluded. The vessel will collapse slowly on the side toward which the blood flows. Then the needle, held almost parallel to the vein, is slipped along in the direction of the current until the point enters the vessel. The egg may be tilted to secure a better position for the needle, if necessary. When the needle has entered, a little of the material forced from the syringe may be clearly seen through the transparent vessel wall, flowing

TABLE VIII
Tests for Hemolysin after Intravenous Injection

Time after injection *	Blood serum	Chorio-allantoic fluid	Amniotic fluid
5 min.	++++	++++	+
2 hrs.	++++	++++	++
4 hrs.	++++	++++	=
8 hrs.	++++	++++	+++
12 hrs.	++++	++++	++
24 hrs.	++++	++++	+
72 hrs.	++++		
6 days	++++	++++	++++

* Each interval refers to tests on 1 embryo.

in the direction of the blood. Arteries are more difficult to enter than veins, but this is not impossible. However, the membranal arteries are smaller than the membranal veins and the shell is therefore less apt to be opened at the position of an artery. The hemorrhage resulting from intravenous injections may sometimes be stopped by lifting the vessel with the needle to cut off the blood flow and searing the point of hemorrhage with a heated needle. This procedure requires some manual dexterity but can be acquired with practice. Usually the amount of blood lost is not significant.

By this method exact amounts of material can be introduced into the chick embryo, leaving the membranes practically intact. To keep a high percentage of the embryos alive after the injection of so much foreign protein, a few precautions should be taken. The material to be injected should be kept warm. The embryos should be returned to the incubator immediately after injection. About

0.05 cc. seems to be the most satisfactory amount for injection, although some serums seem to be more toxic than others. Five hundredths of a cubic centimeter equals approximately one twelve hundredth of the total weight of the egg.

Eight-day-old embryos received injections by this method with 0.05 cc. amounts of hemolysin and were tested at varying intervals. The results are tabulated in Table VIII.

Thus the experimental introduction of hemolytic amboceptor into the chick embryo has indicated that very little of this foreign antibody is absorbed so as to be demonstrable at the intervals used after dropping it onto the chorio-allantoic membrane or injecting it into the yolk sac.

Only after direct injection into the body of the embryo or into the membranal veins has hemolysin been recovered in significant quantity from the blood and extra-embryonic fluid.

Although a much simpler procedure technically, injection into the embryo itself has the disadvantage that it cannot easily be controlled, and it involves considerable damage to the chorio-allantoic membrane. This is especially disadvantageous should one wish to use the membrane subsequently for inoculation with an infectious agent.

By means of intravenous injection a stable antibody can be introduced into the chick embryo, and in the case of hemolytic amboceptor it can persist relatively undiminished for as long as 6 days. These experiments suggest that hemolytic amboceptor may be excreted, possibly by the kidneys, to gain access to the chorio-allantoic fluid.

Unlike complement, amboceptor, in our experience, seems to remain stable within the tissues of the embryo within the time limits employed, that is through 6 days. Although quantitative measurement has not been made, the tests performed indicate very little loss of amboceptor from the fluids and tissues as a whole.

Injection of Complement and Amboceptor into the Same Embryo

In order to study the combined effects of complement and antibody on infection induced in the chick embryo it would be necessary to introduce both substances simultaneously or at intervals into the same experimental host. It was important to determine in these preliminary experiments that both complement and antibody

can circulate in the blood stream in effective amounts and react together on the antigen under investigation.

To test this possibility hemolytic amboceptor was first injected intravenously, followed later by a similar injection of guinea pig complement. At intervals serum of the embryo was removed and tested for hemolysis against a suspension of sheep cells.

Intravenous Injection of Hemolysin and Complement into the Same Chick Embryos

Three 13-day-old chick embryos received intravenously 0.025 cc. of 1:1000 antisheep hemolysin. Twenty-four hours later each of these embryos also received an intravenous injection of 0.2 cc.

TABLE IX
Combined Intravenous Injection of Complement and Hemolysin

Time elapsed after complement injection	Blood serum	Amniotic fluid
5 minutes	+++	—
1 hour	++	±
2 hours	+	+

fresh guinea pig complement. The 3 chicks were killed in succession at intervals of 5 minutes, 1 hour and 2 hours. Blood serum and amniotic fluid of each were collected. To 0.2 cc. of each of these was added 0.3 cc. of 0.85 per cent saline and 0.1 cc. of 2 per cent red sheep cells. The results are shown in Table IX.

DISCUSSION

Our experiments have confirmed the observations of others that antisheep hemolytic complement is absent from the fluids and tissues of chick embryos. This complement makes its appearance within a day or two after hatching and gradually increases in concentration presumably until maturity.

Because of the absence of complement in the chick embryo, a host proved to be susceptible to a variety of pathogenic infectious agents, it might be possible to discover whether complement necessarily participates in a variety of protective reactions between the host and invading parasites, both by determining whether or not certain reactions take place in a host devoid of complement, and

by the passive introduction of complement preferably by intravenous injection.

The chick embryo has been found to be susceptible to certain infectious agents to which newly hatched chicks are immune. What part complement may play in this greater susceptibility is open to investigation.

It is still a question whether or not antitoxic and antibacterial reactions are in part dependent on the presence of complement; and since it has been shown that antibody contained in foreign serum and complement can be successfully introduced intravenously into embryos, the effects of specific antibodies, with and without complement, can be studied in this living host in relation to combating or preventing the infections to which they are proved to be susceptible.

In the case of the neutralization of diphtheria toxin by antitoxin complement does not seem to be at all necessary.³ But the case might be quite different with antibacterial antibodies, and a relatively uniform host in which these important questions can be studied should be of considerable usefulness. Any direct experimental means of demonstrating and measuring the effectiveness of serums now in use for the prevention or amelioration of infectious disease would be of inestimable value.

It was with the idea of developing such a technique that the present investigation was undertaken. The results will be utilized in testing the effectiveness of antitoxic and antibacterial antibodies passively introduced into the embryo before and after infection with those pathogenic agents that induce in this living host lesions and other pathological phenomena similar to those encountered in natural hosts.

It seems evident that for testing the effectiveness of complement and antibody, whether singly or combined, the intravenous route of introduction of the desired materials is the technique of choice and offers the greatest possibilities. Under these circumstances complement is demonstrable in the circulating blood for several hours, and the more stable hemolytic antibody for several days. This would indicate that the effects of the presence of similar antitoxic or antibacterial antibodies can be determined under conditions of induced infection in this host.

SUMMARY AND CONCLUSIONS

1. Complement for sensitized sheep cells is not present in the serum, extra-embryonic fluids and tissues of the chick embryo before hatching. After hatching it is suddenly present and seems gradually to increase to a maximum in the adult fowl.

2. Neither hemolytic amboceptor nor complement appears regularly in the circulating blood of the chick embryo 24 hours after dropping them onto the chorio-allantoic membrane or after injection into the yolk sac or albumin sac.

3. Following injection of hemolytic amboceptor into tissues of chick embryos it was recovered from the circulating blood or extra-embryonic fluids after 72 hours.

4. Following intravenous injection into chick embryos, hemolytic amboceptor was demonstrated in the serum and extra-embryonic fluids of the embryo over a period of 6 days. Complement disappears in about 16 hours after this mode of injection and appears in somewhat diminished quantity in the chorio-allantoic fluid after its disappearance from the blood stream.

5. Amboceptor and complement, circulating concurrently after intravenous injection into a chorio-allantoic vein, were proved to be effective in hemolyzing sheep red cells for a period of at least 2 hours.

6. The method of intravenous injection as described is recommended for the study of the effect of immune substances on subsequent infections of the chick embryo.

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THE FAILURE OF ALLERGIC INFLAMMATION TO PROTECT RABBITS AGAINST INFECTION WITH VIRULENT PNEUMOCOCCI *

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Although inflammation is generally regarded as a protective mechanism of great importance, opinion still differs concerning the actual part it plays in bacterial localization. Some workers believe that the localization is accomplished by the formation of a mechanical barrier from the rapid coagulation of plasma and thrombosis of lymphatic vessels. Therefore, unless the bacteria give off substances that hinder this formation, the effectiveness of the localizing process should vary directly with the speed and intensity of the inflammatory reaction, and allergic inflammation, because of its accelerated development and greater intensity, should tend to localize bacteria near the portal of entry more rapidly and more effectively. This mechanical "walling off" theory has been widely accepted, particularly in the field of tuberculosis, and the belief is general that allergic inflammation causes a more effective walling off of tubercle bacilli in reinfection.

Other workers question the primary importance of a mechanical barrier in bacterial localization. They suggest, rather, that the deposition of fibrin and the thrombosis of lymphatics is essentially a secondary phenomenon accompanying the inflammatory response. The primary factor in the localization is the interaction of the bacteria and the tissues whereby the bacteria either fail to grow and spread freely, or, as in acquired immunity, tend to adhere to one another and to the tissues. The inflammatory reaction, through the local accumulation of leukocytes and immune substances, helps to reinforce the process of bacterial localization and hinders the bacterial growth and spread through the tissues.

Disagreement concerning these two points of view may be due to different experimental methods or to the fact that a biological mechanism adequate in some situations may be totally inadequate in others. For example, an inflammatory reaction which may restrict the growth and spread of slightly virulent or invasive micro-

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organisms may fail to do so with highly virulent ones. Reliance on inflammation as a primary localizing mechanism presupposes its general utility, however, inasmuch as the nature and virulence of microorganisms cannot usually be foretold.

In many of the experiments that have been performed to ascertain the function of inflammation in bacterial localization, bacteria of various kinds were injected into well developed areas of inflammation. Such a procedure, however, cannot prove that inflammation effectively localizes bacteria because it does not show what happens in infections in essentially normal tissues; satisfactory proof would require its production at the time or shortly after the bacteria enter the tissues.

The present paper describes experiments performed to test the assumption that the inflammatory reaction, *per se*, can protect a susceptible animal (rabbit) against infection with a virulent microorganism (*Pneumococcus* type I), and to determine whether the intensified inflammatory reaction of allergy is comparable to the inflammatory response of acquired immunity in protecting an animal against an otherwise lethal infection.

Comparatively few studies have been reported with these questions in mind. Hanger¹ sensitized rabbits to egg albumin and observed that when a suspension of *Bact. leprisepticum* was injected into an area of hypersensitive inflammation, the resulting lesion was intense and "the animals practically always succumbed." When the same type of experiment was performed in rabbits immune to *Bact. leprisepticum*, the local lesions extended "to the border of the hypersensitive reaction, but no further."

Rich² found that if only a few fowl cholera bacilli were suspended in pneumococcal soluble specific substance and injected into the skin of rabbits allergic to it, the animals died of septicemia just as rapidly as did the normal controls. In other words, allergic inflammation developed too slowly to prevent bacterial spread and death of the animals. Bull and McKee³ observed that if rabbits allergic to pneumococcus were infected intranasally with a broth culture of *Bact. leprisepticum*, followed later by the production of a local allergic inflammation in the nose by instillation of pneumococcic autolysate, the infection flared up and the animals died from a blood stream invasion by *Bact. leprisepticum*. These experiments all indicate that a violent allergic inflammation occurring at a portal

of entry may tend to disseminate the infectious agents rather than to localize them.

The experiments of Opie⁴ on the nature of the Arthus reaction suggest that this phenomenon is due to the union in the tissues of antigen and antibody, accompanied or quickly followed by an accelerated inflammatory response which tends to keep the protein localized or fixed. If this interpretation is correct, it should be possible, by suspending virulent microorganisms in a solution of protein, to determine whether or not the accelerated inflammatory response of the Arthus reaction might also localize the bacteria. If allergic inflammation is an important primary mechanism of defense in early infection, such a procedure should demonstrate this fact. Also, its influence on the growth of the bacteria in the field of inflammation and on their spread to the body as a whole could be observed. With this object in mind the following experiments were performed.

MATERIALS AND METHODS

Rabbits weighing from 1600 to 2000 gm. were prepared as follows: One group was sensitized to egg albumin by the subcutaneous injection, at intervals of from 5 to 7 days, of 1 cc. of a 5 per cent aqueous solution of powdered egg albumin. Positive Arthus reactions appeared usually after from 6 to 7 injections. These animals are referred to as allergics,* and were used in the experiments from 7 to 10 days after the last injection. A second group of animals was prepared similarly, but during the course of sensitization the rabbits were also immunized against the pneumococcus with a formol-killed culture of pneumococci or of a pneumococcic autolysate. These animals are referred to as allergic-immunes. A third group of rabbits was immunized against the pneumococci, but was not sensitized to egg albumin; these animals are called pneumococcus-immunes. The fourth group served as normal controls.

The pneumococci for the experiments were grown for from 6 to 8 hours in dextrose rabbit serum broth; sterile broth was added to the bacterial suspension so that dilutions of from 10^{-1} cc. to 10^{-8} cc. of the original culture were present in a volume of 0.5 cc. These

* The term allergy as used in this paper is employed in its broadest sense of altered reactivity with the locally accelerated development of hyperergic inflammation.

dilutions were then mixed carefully with equal volumes of 10 per cent egg albumin solution and 1 cc. of the mixture was injected subcutaneously. In some of the experiments the viable germ-content of the higher dilutions was determined by plating in blood agar (poured plate method) 0.5 cc. of the pneumococcic dilution. The *Pneumococcus* type I used was culture A5, which was obtained from Dr. O. H. Robertson.⁵ This microorganism, under appropriate conditions, will kill rabbits almost invariably in a dilution of 10^{-7} cc.

THE COMPARATIVE PROTECTIVE VALUES OF NORMAL AND ALLERGIC INFLAMMATION AND OF ACTIVE SPECIFIC IMMUNITY IN PNEUMOCOCCIC INFECTION

Series 1:

Ten rabbits allergic to egg albumin and 10 normal controls were injected subcutaneously with the albumin-pneumococcus mixture, the dilutions of the pneumococcic culture ranging from 10^{-2} cc. to 10^{-7} cc. The allergic animals all appeared healthy, being survivors of a group of 18 which had been injected with egg albumin over a period of several weeks. During this time 5 of the animals died, 1 failed to become Arthus-positive, and 2 were not used because of illness at the time of the experiment. These 10 survivors, therefore, were presumably somewhat hardier than the control animals with which they were compared. As a check on the influence of active immunity in general protection, 5 animals previously immunized with the pneumococcic vaccine were injected intravenously with dilutions of culture ranging from 10^{-3} cc. to 10^{-7} cc. The results of this experiment are shown in Table I.

The inflammatory lesions in the allergic animals were all visible within from 3 to 6 hours and at the time of death were edematous, indurated, and in most cases contained hemorrhagic centers. Those in the normal rabbits were typical of the dermal pneumococcic lesion, with a diffusely spreading edema and small hemorrhagic spots in the skin, but with no induration or focal hemorrhagic center. It is obvious that an intense allergic inflammatory reaction failed in every instance to prevent death, regardless of any part it may have played in favoring bacterial fixation at the portal of entry. In other words, inflammation had no appreciable influence on the ultimate outcome of the infection, either in time or degree.

On the other hand, the survival of 4 of the 5 immune animals after intravenous injection indicates the superiority of general specific immunity over local inflammation in a non-immune animal as a life-saving procedure.

TABLE I

The Comparison of the Lethal Effect Following the Subcutaneous Injection of Pneumococcus-Egg Albumin Mixture in Normal, in Egg Albumin-Allergic, and in Pneumococcus-Immune Rabbits

Animal No.	Dilutions of pneumococcus culture	Fate of animals: hours until death	Heart's blood cultures
<i>Allergic</i>			
1	10^{-2}	16	o
2	10^{-3}	36	+
3	10^{-3}	24	+
4	10^{-4}	24	+
5	10^{-4}	24	+
6	10^{-5}	24	+
7	10^{-5}	36	+
8	10^{-6}	48	+
9	10^{-6}	56	o
10	10^{-7}	36	+
<i>Non-allergic</i>			
11	10^{-2}	36	+
12	10^{-3}	24	+
13	10^{-3}	20	+
14	10^{-4}	18	+
15	10^{-4}	30	+
16	10^{-5}	20	+
17	10^{-5}	36	+
18	10^{-6}	18	+
19	10^{-6}	36	o
20	10^{-7}	24	+
<i>Pneumococcus-immune</i>			
21	10^{-3}	Lived	
22	10^{-4}	24	o
23	10^{-5}	Lived	
24	10^{-6}	Lived	
25	10^{-7}	Lived	

o = Cultures not taken.

Series 2:

A smaller group of 6 rabbits (2 allergic, 2 immune to the pneumococcus alone, and 2 normals) were injected subcutaneously with the albumin-pneumococcus mixture in which the pneumococci were in high dilution (10^{-5} cc. to 10^{-8} cc.). The allergic animals gave strong Arthus reactions 9 days before, and the pneumococcus-

immune rabbits had survived an intravenous injection of 10^{-4} cc. of pneumococcic culture a week previously. The results (Table II) show that only the animals immunized against the pneumococcus survived and in them the local lesion at the end of 24 hours was practically negligible.

In both series, therefore, allergic inflammation failed to protect the host by walling off the bacteria before they could enter the blood stream. The actively immunized animals, with one exception, remained healthy even when injected with from 10 to 100

TABLE II

The Comparison of the Lethal Effect from the Subcutaneous Injection of Pneumococcus-Egg Albumin Mixture in Normal, Egg Albumin-Allergic, and Pneumococcus-Immune Rabbits

Animal No.	Dilutions of pneumococcus culture	Fate of animals: hours until death	Remarks
<i>Allergic</i>			
26	10^{-7}	48	
27	10^{-8}	48	
<i>Normal</i>			
28	10^{-7}	48	
29	10^{-8}	48	
<i>Pneumococcus-immune</i>			
30	10^{-5}	Lived	Minimal local inflammation
31	10^{-6}	Lived	Minimal local inflammation

All animals injected subcutaneously.

times more pneumococci than the non-immunes. Furthermore, the local inflammatory lesions in the 2 immune animals were minimal in size at the time that the normal and allergic animals died. There was no correlation between the extent or intensity of the local inflammation and the protection of the host. Practically, therefore, neither the vigor of the inflammatory reaction nor its ultimate effect in immobilizing the bacteria would seem to matter if ultimately the animal dies from an overwhelming bacteremia. Evidently, in any situation in which the escape of only a few virulent bacteria may lead to a fatal outcome, little confidence can be placed in inflammation as a primary determining element in a mechanism of defense.

THE PROTECTIVE EFFECTS OF EXISTING INFLAMMATION IN
NORMAL, ALLERGIC AND IMMUNE RABBITS INFECTED
WITH VIRULENT PNEUMOCOCCI

Inasmuch as many workers have attempted to determine the value of inflammation as a localizing mechanism by the inoculation of bacteria into previously prepared areas of inflammation, we next injected small numbers of virulent pneumococci directly into areas of developing inflammation produced in normal, albumin-allergic, and pneumococcus-immune rabbits. Six rabbits (2 allergic, 2 pneumococcus-immunes and 2 normals) were injected subcutaneously with 1 cc. of a 5 per cent solution of egg albumin. Later (2 hours in one group and 3 hours in the other), 10^{-7} cc. of pneumococcic culture were injected through the original needle path into the field of developing inflammation. Cultural tests of this dilution yielded 56 colonies of pneumococcus. The results (Table III) show that

TABLE III

The Comparative Resistance of Allergic, Immune, and Normal Rabbits to Subcutaneous Injections of Virulent Pneumococci into a Developing Inflammatory Focus

Animal No.	Age of inflammation in hours	Dilution of pure culture of pneumococcus	Fate: hours until death
32 Egg albumin-allergic	2	10^{-7}	48
33 Pneumococcus-immune	2	10^{-7}	Lived
34 Normal	2	10^{-7}	72
35 Egg albumin-allergic	3	10^{-7}	72
36 Pneumococcus-immune	3	10^{-7}	Lived
37 Normal	3	10^{-7}	48

neither normergic nor allergic inflammation of from 1 to 3 hours duration prevented bacterial generalization and death. The pneumococcus-immune rabbits, however, showed no ill effects from the injection, demonstrating again the superiority of active specific immunity over non-specific inflammation as a protective mechanism.

MORPHOLOGICAL FINDINGS IN LESIONS OF NORMAL, ALLERGIC,
ALLERGIC-IMMUNE, AND IMMUNE RABBITS FOLLOWING THE
SUBCUTANEOUS INJECTION OF VIRULENT PNEUMOCOCCI

Finally, 13 rabbits were injected subcutaneously with the albumin-pneumococcus mixture in order to secure tissues for histological examination. Three animals allergic to egg albumin, 3 both

allergic to egg albumin and immune to pneumococcus, 4 immune to the pneumococcus alone, and 3 normals received dilutions of pneumococcic culture suspended in egg albumin ranging from 10^{-5} cc. to 10^{-8} cc. Cultures from 10^{-8} cc. yielded only 4 colonies of pneumococcus. The results are shown in Table IV. The comparison of the survival time in this series is not strictly accurate inasmuch as the survivors were sacrificed in order to obtain tissues comparable to those of the animals that died spontaneously.

TABLE IV

The Comparative Resistance of Normal, Egg Albumin Allergic, Pneumococcus-Immune, and Egg Albumin Allergic-Pneumococcus Immune Rabbits to Subcutaneous Injection of Virulent Pneumococci

Animal No.	Amount of culture in cc.	Fate of animals: hours until death	Remarks
38 Allergic	10^{-8}	48	
39 Allergic	10^{-8}	48	
40 Allergic-immune	10^{-8}	Lived	Killed for sections
41 Allergic-immune	10^{-8}	Lived	Killed for sections
42 Immune	10^{-8}	Lived	Killed for sections
43 Normal	10^{-8}	72	
44 Allergic	10^{-7}	48	
45 Allergic-immune	10^{-7}	Lived	Killed for sections
46 Immune	10^{-7}	Lived	Killed for sections
47 Normal	10^{-7}	48	
48 Immune	10^{-5}	Lived	Killed for sections
49 Immune	10^{-5}	Lived	Killed for sections
50 Normal	10^{-5}	48	

Nevertheless, there is the same general trend as in the three previous series. All of the animals immune to the pneumococcus and those both immune to pneumococcus and allergic to egg albumin were alive and healthy at the time when the normal rabbits and those allergic to egg albumin had died. It is obvious, again, that allergic inflammation failed to prevent a fatal outcome even when only a few pneumococci, probably not more than from 5 to 10 microorganisms, were injected.

The tissues were fixed in Zenker's solution with 5 per cent glacial acetic acid, were embedded in celloidin, and the sections were stained with hematoxylin and eosin, hematoxylin-eosin azure, and with the Gram stain by the Wallace modification.⁶ The findings will be described briefly for each group.

those seen in a positive Neufeld reaction (Fig. 3). In some instances isolated colonies of pneumococci were also present in acellular areas (Fig. 4). Small clumps of adherent swollen microorganisms indicated an agglutinative process. Phagocytosis was not particularly conspicuous at the borders of these areas.

Local Lesions in Rabbits Immune to Pneumococcus

Here there was but little evidence of inflammation and pneumococci were exceedingly difficult to find in the field of inflammation although they could be seen occasionally within leukocytes. The bacteria had evidently been engulfed so quickly after their entrance into the tissues that very little bacterial proliferation occurred; consequently, very little inflammation ensued (Fig. 5).

DISCUSSION

An extensive discussion of these experiments is unnecessary inasmuch as we were not concerned with the general problem of allergy and its relation to resistance, but only with the ability of an allergic inflammatory reaction to restrict the growth and spread of virulent bacteria. Neither were we concerned with the relation of bacterial invasiveness to inflammation.⁸ We wished only to ascertain whether a local allergic inflammation may hinder the escape of unquestionably virulent microorganisms from the region of developing infection. Our experiments indicate that any such hindrance is negligible.

The most conspicuous feature of our investigation was the uniformity and regularity with which both ordinary and allergic inflammation failed to prevent the escape of virulent pneumococci from their site of lodgement, both in non-inflamed tissues and in those undergoing early inflammation. Every normal and allergic animal died with no significant difference in the length of infection time. Histological examination of the local lesions in the allergic animals showed, furthermore, that conditions favorable for the local coagulation of plasma, thrombosis of lymphatics and accumulation of leukocytes failed either to prevent abundant proliferation of the bacteria or to modify their spread to the body as a whole. This is not surprising, however, as there is no reason to suppose that bacteria able to grow in the body fluids and within the leukocytes of a susceptible animal should be influenced adversely by a

greater accumulation of such fluids and cells. Vorwald,⁹ in fact, has shown that if equal numbers of tubercle bacilli are injected into an animal of high resistance (cat) and one of low resistance (guinea pig), the intensity of the inflammatory reaction varies inversely with the degree of resistance rather than directly. Nor is it surprising that inflammation fails as a defensive mechanism under conditions in which bacteria can proliferate so abundantly in the inflammatory exudate itself.

The uniformity with which the pneumococci in the allergic lesions disseminated raises the question whether the edema and necrosis accompanying the inflammation did not favor bacterial growth and spread. Even in the rabbits both allergic to egg albumin and immune to the pneumococcus, bacterial growth was more abundant than in those only immune to the pneumococcus. Field, Drinker and White¹⁰ have demonstrated the early increase in lymph flow from an area of inflammation, and Rhoads and Goodner⁷ observed that pneumococci in the dermal lesions of rabbits reach the adjacent tissues by the edema fluid rather than because of their inherently invasive properties. Angevine^{11, 12} showed that when hemolytic streptococci, either virulent or relatively avirulent, are injected intradermally into rabbits sensitized or immunized to them, they multiply more rapidly for several hours and persist longer in the local lesions than in the lesions of the corresponding controls. He concluded that "in sensitized animals local injury with necrosis favors the multiplication of relatively avirulent streptococci at the site of entry and explains their survival at a time when they have disappeared in the controls." Lurie¹³ more recently has found that when he injected tubercle bacilli suspended in melted agar into normal and tuberculous rabbits, the microorganisms spread more rapidly to the regional lymph nodes of the tuberculous (allergic) than to those of the normal animals. It is evident from these experiments, as well as from our own, that rather than acting as an effective localizing agency, the allergic inflammation may actually increase the local vulnerability.

Another significant finding was the character and extent of the growth of the pneumococci in the different types of local lesions, particularly in the rabbits both allergic to egg albumin and immune to the pneumococcus. The presence of many large pneumococcic colonies as long as 40 hours after the injection of from approxi-

mately 5 to 50 pneumococci reemphasizes the fact that extracellular antibacterial substances exert but slight effect upon Gram-positive microorganisms.¹⁴ Nevertheless, an extracellular antibody-effect exists, as evidenced by the enlarged diplococci with greatly swollen capsules (Fig. 3). This change is due, presumably, to the prolonged action of immune substances upon the bacterial surfaces, thus causing them to become sticky and to adhere to one another and to the tissues. The not infrequent occurrence of small clumps of these swollen bacteria indicates, furthermore, that increased cohesiveness has also initiated the process of agglutination in the tissues.

The insignificant local lesions in the rabbits immune to the pneumococcus, the histological evidence of only small foci of leukocytes in which pneumococci were almost absent, and an absence of any significant degree of fibrin deposition or thrombosis of lymphatics reinforces the general argument of this paper that inflammation, *per se*, plays no essentially determining part in the primary localization of virulent bacteria. It is altogether likely that when virulent bacteria enter immune tissues in the small numbers introduced in these experiments they are localized so sharply and engulfed so quickly that there is very little stimulus for the outpouring of any considerable amount of inflammatory exudate, so that at the end of from 36 to 40 hours the gross lesion is practically invisible and the microscopic findings are minimal.

SUMMARY AND CONCLUSIONS

Experiments were performed in an attempt to ascertain to what extent the accelerated and intense inflammatory reaction of allergy can act as a protective mechanism in a susceptible animal infected with a highly virulent microorganism. Fifty rabbits were used as follows: Some were sensitized to egg albumin by a series of subcutaneous injections until they became Arthus-positive; others were similarly prepared but were also immunized against a type I pneumococcus by injection of formol-killed cultures; a third group was immunized in the same way against the pneumococcus but was not sensitized to egg albumin, and a fourth group served as a control. The essential experiments consisted in mixing various dilutions of young broth cultures of the pneumococcus with egg albumin, injecting the mixture subcutaneously, and ob-

serving the development of the inflammatory lesion, the histopathological changes occurring therein, and the fate of the animal. The results may be summarized as follows:

1. As few as 4 pneumococci when suspended in 5 per cent egg albumin and injected subcutaneously caused the death within from 36 to 40 hours of all of the normal rabbits as well as those allergic to egg albumin. Allergic inflammation, therefore, failed to protect the rabbit against infection with virulent microorganisms. The pneumococci, furthermore, grew profusely in the areas of allergic inflammation, and spread evenly through the edematous field of inflammation. Phagocytosis, although moderately active, did not modify the course of the infection either in time or degree, and the pneumococci grew freely in the exudate itself. In other words, local conditions in the field of inflammation had no determining influence ultimately on the course of the infection.

2. The pneumococci also grew freely for a time in the lesions of rabbits both allergic to egg albumin and immune to the pneumococcus, but the microorganisms tended to remain localized near their sites of introduction. Here they developed into isolated colonies or became swollen, with prominent capsules, suggesting the appearance of a positive Neufeld reaction. Many of them lost their Gram-positive characteristics, presumably as a result of the extracellular action of antipneumococcic immune substances in the edema fluid. The animals, however, suffered no serious effects from the infection.

3. The lesions in actively immunized rabbits were small and inconspicuous after from 36 to 40 hours. Leukocytes were assembled in clusters in the area of infection but contained very few visible pneumococci, indicating the superiority of specific immunity over non-specific inflammation, whether normergic or allergic, in restricting the growth and spread of the microorganisms through the tissues.

4. These experiments, therefore, offer no support to the view that an inflammatory reaction can develop quickly enough to prevent the escape of virulent bacteria from their site of lodgement in previously non-inflamed tissue and cast further doubt on the validity of the hypothesis of a mechanical "walling off" as an important mechanism for bacterial fixation in a non-immune animal.

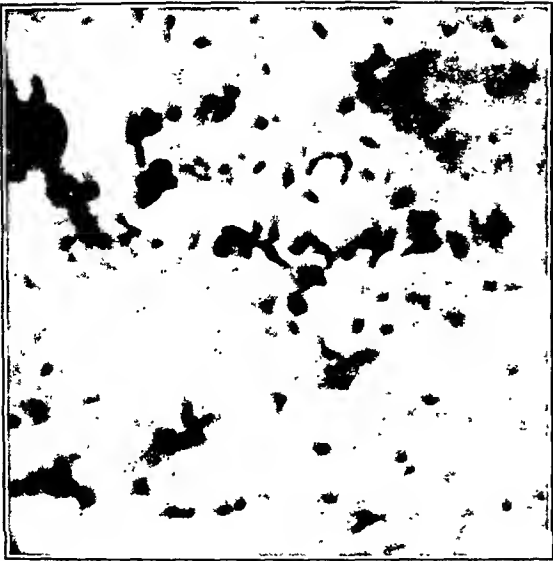
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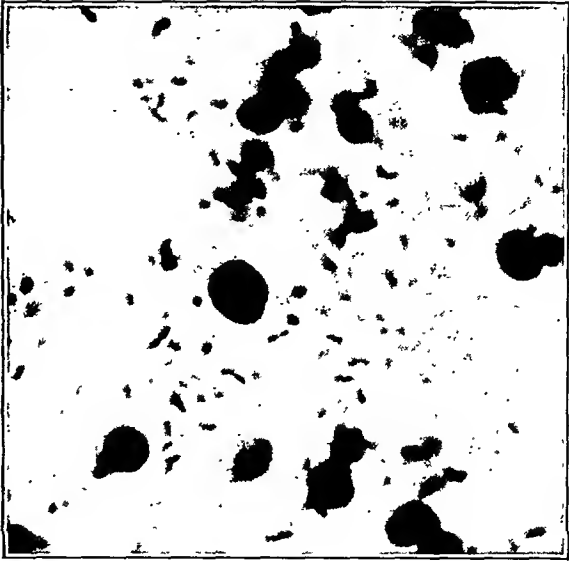
DESCRIPTION OF PLATE

PLATE 19

- FIG. 1. Area of subcutaneous inflammation in a normal rabbit injected with 10^{-7} cc. of pneumococcic culture. Death in 36 to 40 hours. Note the large numbers of pneumococci which have proliferated freely and have spread uniformly through the area of inflammation. Note also the absence of leukocytic infiltration. Gram stain (Wallace modification). $\times 2000$.
- FIG. 2. Area of subcutaneous inflammation in a rabbit allergic to egg albumin and injected with 10^{-8} cc. of pneumococcic culture (approximately 4 pneumococci). Death in 36 to 40 hours. Note the large numbers of pneumococci which have multiplied and spread diffusely despite a marked infiltration of leukocytes. Gram stain (Wallace modification). $\times 2000$.
- FIG. 3. A portion of the field of inflammation in a rabbit both allergic to egg albumin and immune to pneumococcus. Injected subcutaneously with a pneumococcus-egg albumin mixture containing 10^{-8} cc. of pneumococcic culture. Rabbit sacrificed in 42 hours. Note the greatly swollen pneumococci and the prominent capsules. Gram stain (Wallace modification). $\times 2000$.
- FIG. 4. Section of a subcutaneous lesion in a rabbit both allergic to egg albumin and immune to pneumococcus and injected with egg albumin-pneumococcus mixture containing 10^{-8} cc. of pneumococcic culture (approximately 4 microorganisms). Animal sacrificed in 42 hours. Note the colonies of pneumococci which have developed despite the fact that the animal had been specifically immunized. Gram stain (Wallace modification). $\times 310$.
- FIG. 5. Area of subcutaneous inflammation in a rabbit specifically immunized and injected with the egg albumin-pneumococcus mixture containing 10^{-5} cc. of pneumococcic culture (approximately 7000 pneumococci). Animal sacrificed in 44 hours. Note the large numbers of leukocytes and the almost complete absence of pneumococci. Gram stain (Wallace modification). $\times 2000$.



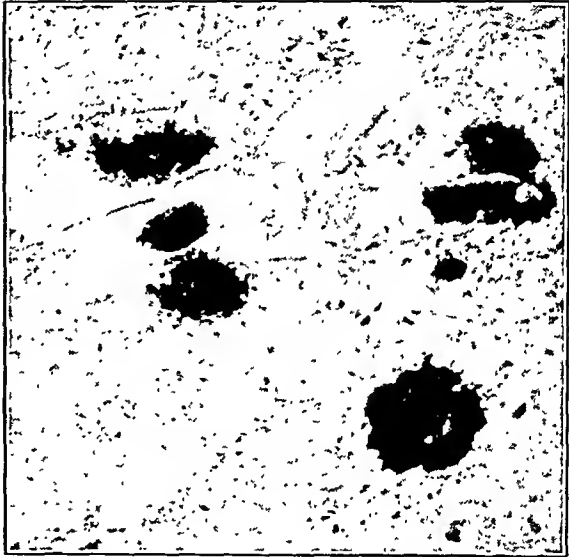
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FATTY INFILTRATION AND CIRRHOSIS OF THE LIVER IN DEPANCREATIZED DOGS MAINTAINED WITH INSULIN *

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It has been shown in this laboratory that completely depancreatized dogs treated with insulin survive for long periods when maintained on a diet adequate in calories, proteins, salts and vitamins, but lacking pancreas.¹ Under such conditions a number of pathological changes appear in the tissues of these animals. Bilateral cataracts have been found as early as 1 year following pancreatectomy.² A disturbance has also been found in the blood lipids: all constituents, in particular cholesterol esters, have markedly fallen soon after excision of the gland.³ The most striking change occurs in the liver, in which large amounts of fat are deposited. These fat changes in blood and liver appear in the absence of pancreas in the diet, for by the addition of pancreas the fall in blood lipids, as well as the accumulation of fat in the liver, can be prevented.⁴

For the present report, the anatomical changes associated with fatty livers have been examined at various intervals following pancreatectomy. Although fatty livers may appear early and remain for long periods following excision of the pancreas, a regression in the fat content of the liver finally occurs. In 3 dogs that survived between 4 and 5.5 years the fat content of the liver returned to levels close to normal. During this entire period of observation the livers of the 49 dogs examined showed two types of lesions. The first of these occurred in association with the early infiltration of fat, while the second or final stage appeared most characteristically in those livers in which the regression of fat had taken place. Such livers showed an extensive periportal fibrosis with irregular lobulation indicative of cirrhosis. The depancreatized dog thus provides a new method for the production of

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experimental cirrhosis, this occurring as a final stage in response to a fatty infiltration of long standing in the liver.

EXPERIMENTAL

The preparation and treatment of the depancreatized dogs employed in this study have been described elsewhere.¹ After pancreatectomy each dog received twice daily a mixture containing meat, sucrose and bone ash. Vitamin supplements (A and D as cod liver oil *; the B complex in the form of a concentrate obtained from rice bran **) were added to the diet mixture twice a week. Each dog received 16 units of insulin daily, 8 units at each time of feeding.

A careful search for pancreatic tissue was made in all depancreatized dogs at autopsy. The completeness of pancreatectomy in all animals recorded in this study was verified.

The autopsies were performed immediately after the animals were sacrificed or within a few hours after death. A complete autopsy was performed in every case, and blocks of tissue were removed from all organs for histological study. After the blocks were removed from the liver for histological examination, the rest of the organ was immediately ground and thoroughly mixed, and samples were taken for determinations of total fatty acids, cholesterol and phospholipids. The methods employed for lipid analyses have been described elsewhere.⁴

I. THE FATTY CHANGES IN THE LIVER AFTER PANCREATECTOMY

Although, as already pointed out, large amounts of fat may appear in the liver soon after pancreatectomy, extremely fatty livers are not of constant occurrence in the early periods. Not only the degree of lipid infiltration, but also the time of onset of such changes show considerable variation. Thus, mixed samples of the entire livers obtained from 2 dogs that received the same treatment contained 32 and 11 per cent of fatty acids at an interval of 3.5 weeks after pancreatectomy. Equally significant variations were found at other intervals. In a series of 30 dogs, however, it

* The standardized cod liver oil used in this study was kindly furnished in part by Mead Johnson and Company, Evansville, Indiana.

** The rice bran concentrate was kindly furnished by Vitab Products, Inc., Emeryville, California.

was found that fatty acids in excess of 14 per cent were present at an interval of 20 to 30 weeks after excision of the gland.

In the normal dog's liver the lobules are not outlined by limiting strands of connective tissue and there is an extremely scanty amount of connective tissue associated with the portal triads. The finer biliary radicles are usually difficult to demonstrate. The central veins can be distinguished: they are situated at regular distances from the portal triads. The cords of hepatic cells are evenly arranged, and the sinusoids radiate uniformly from the portal triads to the central veins. The hepatic cells occasionally contain a small vacuole which is shown by a scharlach R stain to be fat. By chemical analyses such livers have been shown to contain approximately 3 to 4 per cent of fatty acids.

Microscopic Appearance of Livers Containing Various Amounts of Fat Obtained from Depancreatized Dogs in Good Condition

The livers were removed at various intervals after pancreatectomy. In livers containing relatively small amounts of lipids (about 10 per cent), the fat was deposited in large globules replacing the cytoplasm of cells in scattered areas within a lobule (Fig. 1). In some cases the fat globules were irregularly distributed toward the mid portion of a lobule. No change other than the usual displacement of the nucleus was observed in the cells of these livers. In general, the size of the fat globules was uniform.

In livers showing a greater lipid change (approximately 18 per cent), fat was found in almost every cell of the lobule (Fig. 2). The largest globules of fat were generally found in the cells around the centers of the lobules, while the remainder of the cells showed small droplets scattered throughout the cytoplasm. In many of the cells around the portal triads the cytoplasm had become granular and somewhat hyaline in appearance. There were no nuclear changes. The sinusoids were not easily distinguished. The cells of the large bile ducts occasionally contained fat in their cytoplasm.

When about 30 per cent of fat had accumulated in the liver, almost every hepatic cell was completely replaced by fat (Fig. 3). A rare cell, however, retained some cytoplasm which frequently had a hyaline, granular appearance. The sinusoids were completely obliterated and their compressed cells varied in size and

shape. Nuclei may not be present in every cell. There were no changes in the structure of the portal triads. Nearly all the lining epithelial cells of the bile ducts contained fat.

Distribution of Fat in the Liver

The distribution of lipids was studied in the livers of depancreatized dogs in which various degrees of fat infiltration were produced experimentally. Blocks of tissue, varying from 4 to 6 sq. cm. in area and from 3 to 4 mm. in thickness, were removed from each of the lobes for histological examination. Three consecutive slices were removed from each block of tissue. The middle section was used for histological study while the two end slices were combined for chemical analysis.

In livers containing little fat this was distributed in a fairly regular and equal manner throughout the various lobes. Moreover, the sections showed that the fat was evenly distributed within the lobules (Fig. 1). With increasing amounts of fat, however, the various lobes at times showed extreme variations in their fat content (Fig. 2). This was confirmed by chemical analyses. The lobular distribution of lipids likewise varied in these cases; some showed nearly all of the cells filled with fat-stained globules, while others had fat only in the peripheral cells or around the central vein. In a liver containing about 50 per cent of fat the lobes showed very little variation in lipid distribution and the lobules showed all cells uniformly filled with fat (Fig. 3). Thus, while livers that contain either very small or very large amounts of lipids have the fat uniformly distributed, livers in which a moderate degree of lipid infiltration has occurred may show an uneven distribution of fat. The storage and liberation of fat apparently do not occur in a constant or regular manner in the various parts of the liver.

II. EARLY FIBROTIC CHANGES

A second group of livers developed, in addition to the fatty changes noted above, a prominence of the portal spaces caused by a fibrous tissue proliferation that is never seen in the normal liver. The lobulation, however, is not complete, nor is the extensive distortion of the parenchyma so marked as in the cirrhotic livers to be described below. There is some proliferation of the bile ducts. The hepatic cells near the portal fibrous tissue septums generally

show a hyaline granular alteration of the cytoplasm, and occasional cells have undergone marked fatty changes. All these livers show a variable degree of lymphocytic and plasma cell infiltration in association with the growing connective tissue.

1. Dog G8: Female, depancreatized April 2, 1931. This animal survived for 3.3 years after pancreatectomy, at the end of which time it was sacrificed for examination of the liver.

Microscopic Appearance of Liver: The portal spaces were prominent, as there was definite fibroblastic proliferation around them, which gave the liver a definite lobular pattern. The hepatic cells showed extreme variation in fat content, large groups of cells being completely filled whereas adjacent groups had a granular hyaline cytoplasm with no visible fat. The central veins were not easily distinguished (Fig. 4).

2. Dog DJ: Female, depancreatized June 29, 1932. This animal was in good condition until 3 days before Feb. 4, 1934, when it died. The period of survival was 1.6 years. At autopsy an extensive retroperitoneal hemorrhage and cellulitis, which also involved the heart and aorta, were found. Focal hemorrhage and leukocytic infiltration of the right auricular muscle were present.

Microscopic Appearance of Liver: The histological appearance of this liver was similar in all details to that of Dog G8. The terminal acute infectious process had not altered the previous changes in the liver.

3. Dog DG: Female, depancreatized Aug. 1, 1932, died Feb. 14, 1934. Period of survival 1.5 years. This animal refused food for 2 weeks before death occurred. Autopsy revealed an acute urinary tract infection. A mixed sample of the liver contained 29.2 per cent of total lipids.

Microscopic Appearance of Liver: There was a diffuse fatty infiltration in all parts of the liver. The periportal fibrous tissue was arranged in rather thin bands and appeared condensed. Occasional leukocytes, lymphocytes and plasma cells were present in the fibrous tissue. Groups of cells lying against the fibrous tissue septums showed hyaline changes.

4. Dog K: Male, depancreatized Dec. 25, 1930, died Sept. 12, 1933. Period of survival 2.7 years. For several weeks before death this animal lost its appetite and finally refused all food. It was

emaciated at the time of death. At autopsy bronchopneumonia and an acute pyelitis were found. The liver weighed 920 gm. (i.e., 14.2 per cent of the final body weight). A mixed sample of the whole liver contained 33.8 per cent of total lipids.

Microscopic Appearance of Liver: The tendency toward the formation of lobules by a slight periportal fibrosis was seen in all sections. All hepatic cells were well filled with fat, so that the peripherally situated cells rarely showed the hyaline alteration of the cytoplasm. Proliferation of the bile ducts was not distinct. The bile canaliculi occasionally contained plugs of inspissated bile. In the periportal fibrous tissue lymphocytes and plasma cells were rare.

III. CIRRHOSIS OF THE LIVER IN DEPANCREATIZED DOGS

Extensive cirrhosis was found in 4 dogs. In 2 of these (dogs DA and DB) the livers presented the characteristic hob-nailed appearance. The surface was reddish brown in color and was covered by nodules of varying size, the largest of which measured 10 mm. in diameter. The liver on cut section felt sclerotic, and the cut surface showed the parenchyma to be composed of irregular lobules. In the remainder of the animals the surfaces of the livers appeared normal. All livers were enlarged. In all cases the gall-bladder and the extrahepatic bile ducts were normal on gross examination. Bile could be expressed through the papilla by pressure on the gall-bladder. The animals were not jaundiced. All the organs were examined at autopsy and routine sections taken from each one. The protocols of these animals are as follows:

1. DOG DA: Female, depancreatized March 11, 1931. This animal was in good condition at the end of the period of maintenance, Sept. 25, 1936. Its period of survival was 5.5 years. At autopsy the liver was found greatly enlarged and hob-nailed in appearance. It weighed 565 gm., or 7.5 per cent of the body weight. A mixed sample of the liver contained 5.6 per cent of total lipids.

Microscopic Appearance of Liver: A striking amount of fibrous tissue was found around the portal spaces (Fig. 5). These spaces were irregularly distributed, and the fibrous tissue branched from one to another. In this manner the liver parenchyma had been divided into sharply circumscribed lobules that varied extremely in size and shape. Glisson's capsule was greatly thickened. The

central veins were not easily distinguishable and were eccentrically placed. Within the lobules the hepatic cells were arranged in distorted cords compressing the sinusoids. These cells contained a variable amount of fat; in some lobules many cells might contain a large globule of fat, whereas in the adjacent lobule the cells might have very little. Some cells had a markedly vacuolated cytoplasm, whereas others contained a cytoplasm of a granular or hyaline nature. There was a great variation in the thickness of the fibrous septums. Usually there was an associated lymphocytic and plasma cell infiltration, together with a prominent proliferation of the small bile ducts. In the fibrous septums a group of hepatic cells would often be enclosed, and these cells generally showed a condensation of the cytoplasm into hyaline granular masses (Fig. 6).

2. Dog DB: Male, depancreatized July 27, 1932. This animal was in good condition at the end of the period of maintenance, Sept. 25, 1936. Its period of survival after pancreatectomy was 4.2 years. At autopsy the liver was found greatly enlarged and its surface hob-nailed in appearance. It weighed 400 gm., or 5.7 per cent of the body weight. A mixed sample of the entire liver contained 3.5 per cent of total lipids.

Microscopic Appearance of Liver: This was similar in all details to that observed in Dog DA described above.

3. Dog DC: Female, depancreatized Sept. 1, 1931. This animal was in good condition at the end of the period of maintenance, Sept. 29, 1936. Its period of survival was 5.1 years. At autopsy the liver was found enlarged, weighing 480 gm., or 4.5 per cent of the total body weight. A mixed sample of the whole liver contained 3.9 per cent of total lipids.

Microscopic Appearance of Liver: Connective tissue was present in fine radiating strands usually extending outwards from the portal triads in irregular fashion. The lobular pattern could be readily distinguished, but the fibrous tissue septums were not so prominent as in dogs DA and DB. The hyaline alteration of the cytoplasm of the cells at the periphery of the lobule was, however, more striking in this animal than in those described above. Some cells were also undergoing marked shrinkage or showed complete replacement of the cytoplasm by fat.

4. Dog DE: Depancreatized Sept. 5, 1932. This dog was maintained for 2.3 years on the stock diet recorded above, which contained no raw pancreas. For the next 15 weeks it received 250 gm. of raw pancreas daily in addition to the regular stock diet, and at the end of this time it was sacrificed for study of the liver. The total period of survival was 2.6 years, during which the animal was in good condition. The liver was large and weighed 640 gm., or 7.4 per cent of the body weight. A mixed sample of the entire liver contained 4.6 per cent of total lipids.

Microscopic Appearance of Liver: The histological structure was similar to that observed in Dog DC.

In 6 dogs that survived for periods longer than 1 year no cirrhosis or abnormal degree of fibrosis was found in the liver. One of these (Dog DF) was examined 3.1 years after removal of the pancreas, whereas in the 5 other animals (dogs G₁, A₁, A₃, G₃ and G₂) the livers were removed for study at intervals between 1.3 and 1.8 years after pancreatectomy.

DISCUSSION

The results of the present investigation demonstrate the occurrence of cirrhosis of the liver under conditions not hitherto described. Sixteen completely depancreatized dogs were maintained for periods longer than 1 year. The tissues in 14 of these animals were subjected to a careful histological study. Extensive cirrhosis was found in 4 dogs, while in 4 others an abnormal degree of fibrosis was present. Infection seems to play no part in the production of this scarring, for the 4 dogs in which the most marked cirrhosis was found showed no other pathological changes when sacrificed. Incidental acute infections were present in 3 of the second group of 4 dogs. From the appearance of the livers, however, it is obvious that these terminal infections are in no way related to the fibrotic changes that occurred. In all dogs recorded in this study there was no evidence of obstruction in the extrahepatic bile passages at autopsy. The type of lesion produced does not resemble in any particular the changes associated with infection or extrahepatic biliary obstruction.

A constant finding in all these dogs is an early increase in the amount of fat in the liver. This usually takes place in the first few

months after pancreatectomy and remains for long periods. It was shown by chemical analyses that a fatty liver may be present as late as 3 years after pancreatectomy. The fat first appears in scattered cells within a lobule and slowly extends outward from the central veins. The peripheral cells, however, are the first to show cytoplasmic alterations and this is followed by hyaline atrophy of the whole. The process is apparently slow and stimulates avascular fibroblastic proliferation. A few lymphocytes and plasma cells accompany this reaction. Strands of fibrous tissue then intertwine around other peripherally located cells, thus isolating them and making the process a progressive one.

In all livers showing fibrosis some cells can always be found which show extreme hyalinization and granularity of the cytoplasm. These granules may be pushed off to one side if a fat globule is present in the cell. Usually the cells containing this marked cytoplasmic change are arranged in small groups.

The time of onset of these fibrous changes in the liver remains to be considered. No direct relation between the interval after pancreatectomy and the degree of fibrous proliferation was found in the 8 dogs studied. Thus, while all the animals that survived between 4.2 and 5.5 years showed marked cirrhosis, a greater degree of fibrosis was found in 1 dog 2.6 years after pancreatectomy than in another 3.3 years after. No evidence of fibrosis was found in the first few months after pancreatectomy; the earliest signs of such changes occurred after an interval of 1.5 years.

SUMMARY AND CONCLUSIONS

Depancreatized dogs constantly develop fatty livers at variable periods after the operation has been performed. In those kept for from 2.6 to 5.5 years upon an adequate diet and insulin, 8 of 16 developed more or less interlobular fibrosis of the liver associated with a hyaline or colloid degeneration of many cells and hyaline atrophy of peripheral cells. In 4 animals this was so pronounced, both grossly and microscopically, that the picture of a well advanced portal cirrhosis of the liver was present. By the time this severe cirrhosis had occurred, the fat content of the livers had returned to normal and there was little histological evidence that a markedly fatty liver had preceded the fibrosis. The sequence of events appears to be fatty infiltration, hyaline degeneration and

atrophy of cells at the periphery of lobules, and fibroblastic proliferation in orderly fashion, ending with the typical hob-nail appearance and fibrotic structure of cirrhosis. Necrotizing agents introduced from the outside, infection, and extra-hepatic biliary obstruction were excluded as causative factors.

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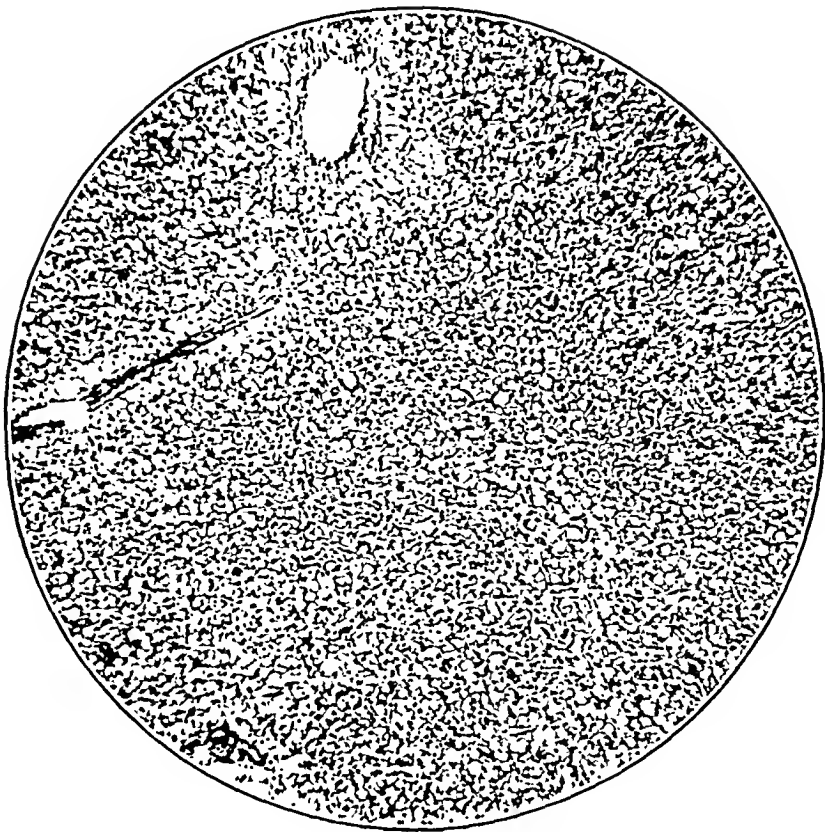
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DESCRIPTION OF PLATES

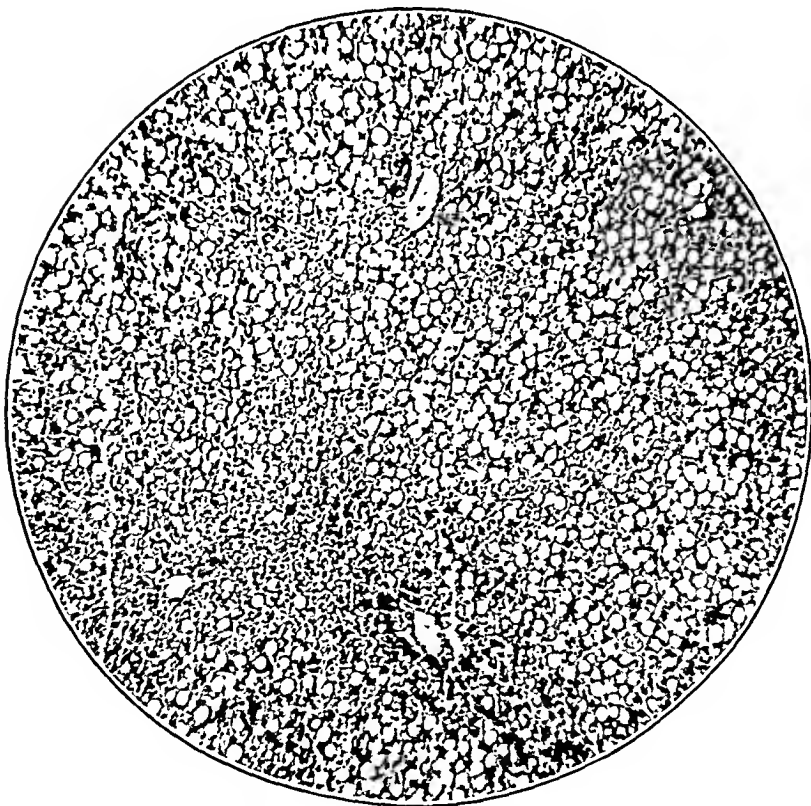
PLATE 20

FIG. 1. Liver of Dog D100A showing early stage of fatty infiltration 3.5 weeks after pancreatectomy. Fatty acid content 10.5 per cent. Hematoxylin-eosin stain. $\times 73$.

FIG. 2. Liver of Dog D94D showing fatty infiltration of intermediate degree in liver 14.5 weeks after pancreatectomy. The liver immediately adjacent to this area contained 17.5 per cent fatty acids. Hematoxylin-eosin stain. $\times 73$.



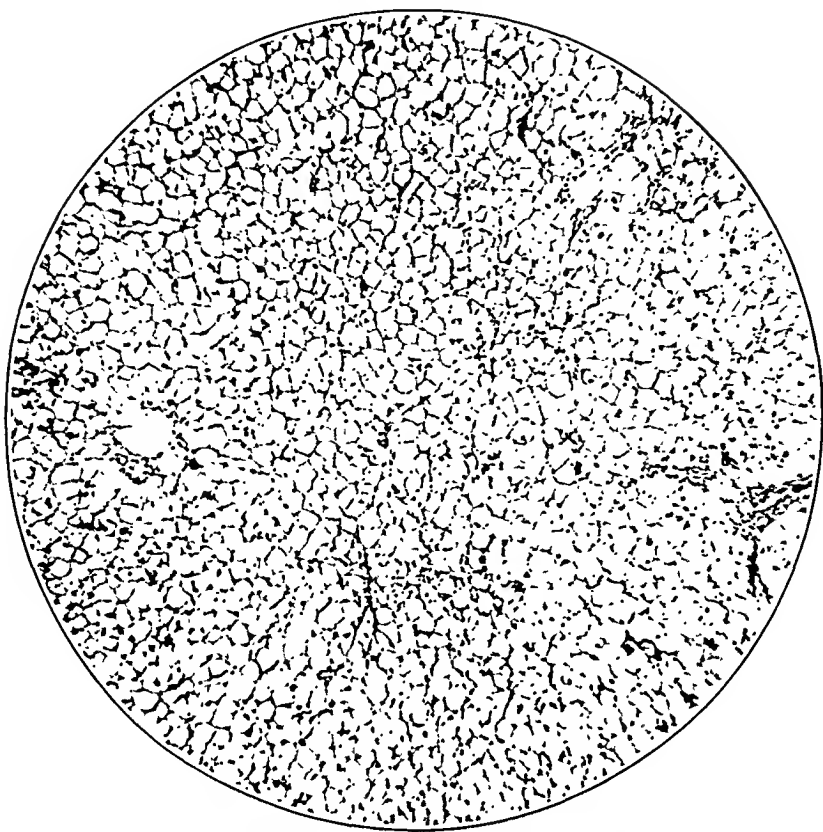
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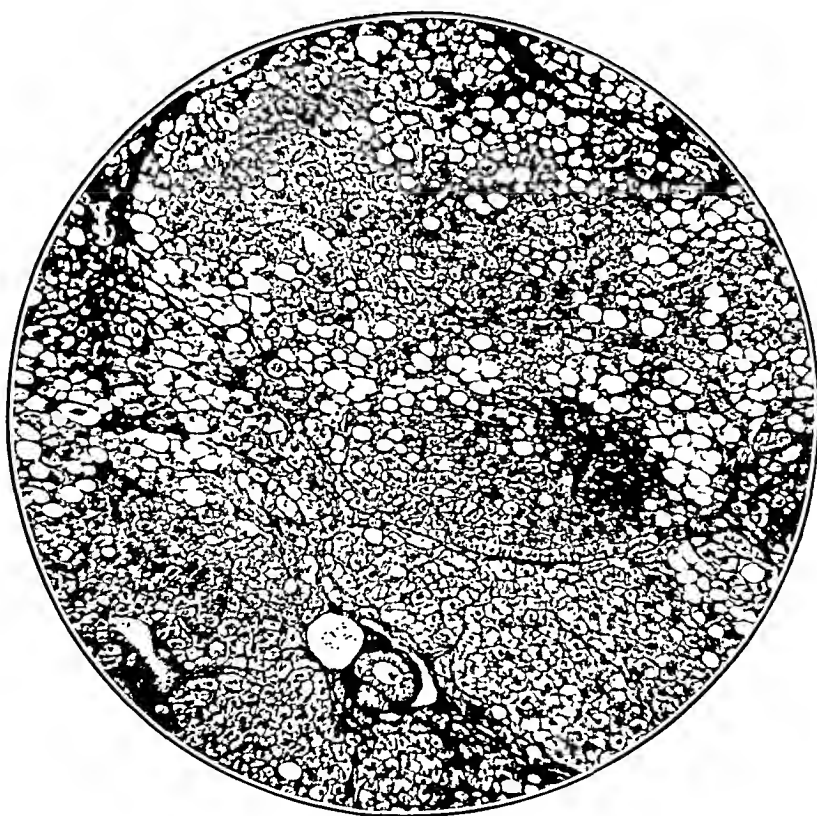
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PLATE 21

- FIG. 3. Liver of Dog D1 showing advanced degree of fatty infiltration 34 weeks after pancreatectomy. A mixed sample of the remaining liver contained 26.3 per cent fatty acids. Hematoxylin-eosin stain. $\times 73$.
- FIG. 4. Liver of Dog G8 showing fibroblastic proliferation with decrease in amount of fat present 3.3 years after pancreatectomy. Phosphotungstic acid hematoxylin stain. $\times 73$.



3



4

PLATE 22

FIG. 5. Liver of Dog DA showing well advanced cirrhosis of the liver 5.5 years after pancreatectomy. There is very little fat present now. Total lipids 5.6 per cent. Phosphotungstic acid hematoxylin stain. $\times 38$.

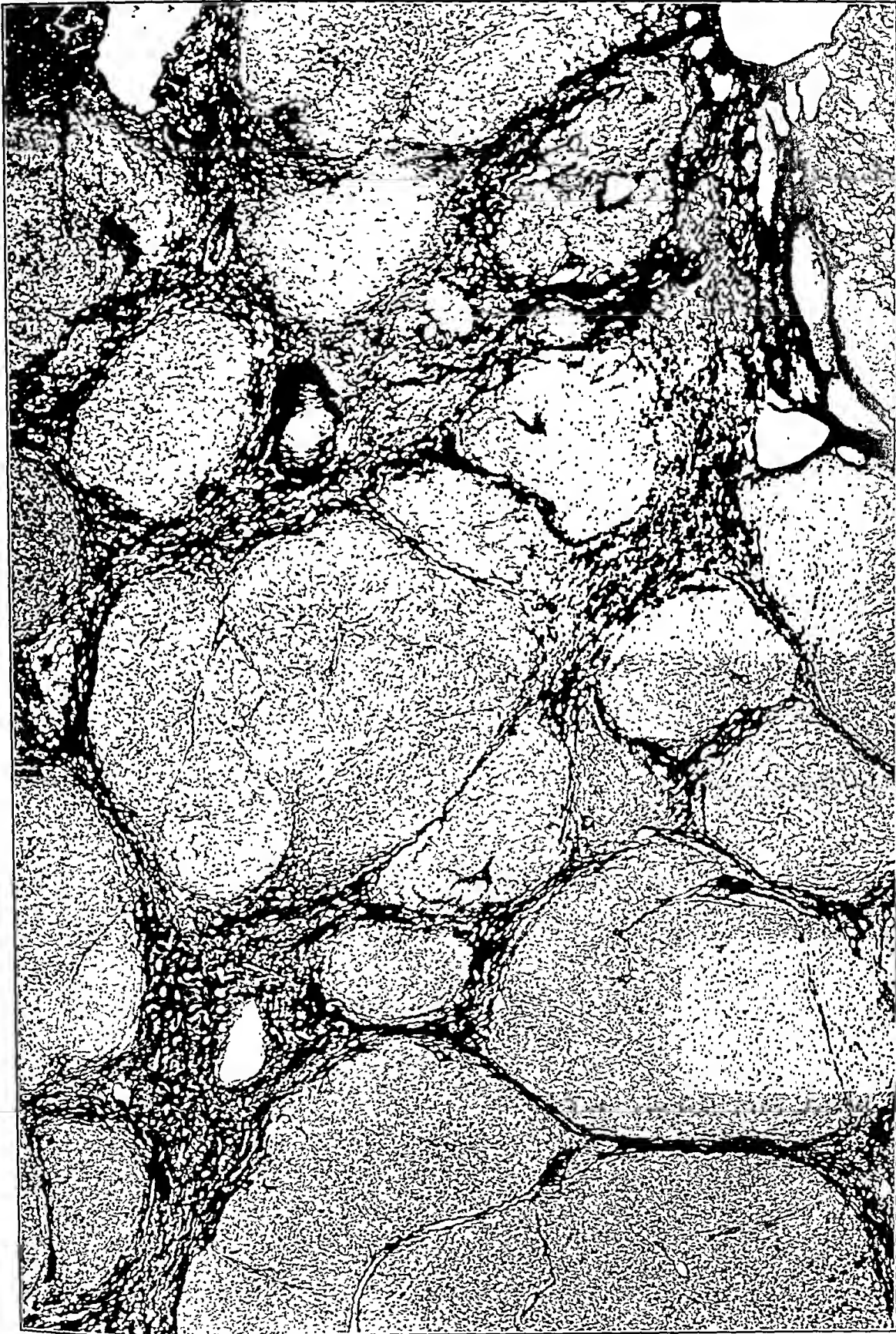
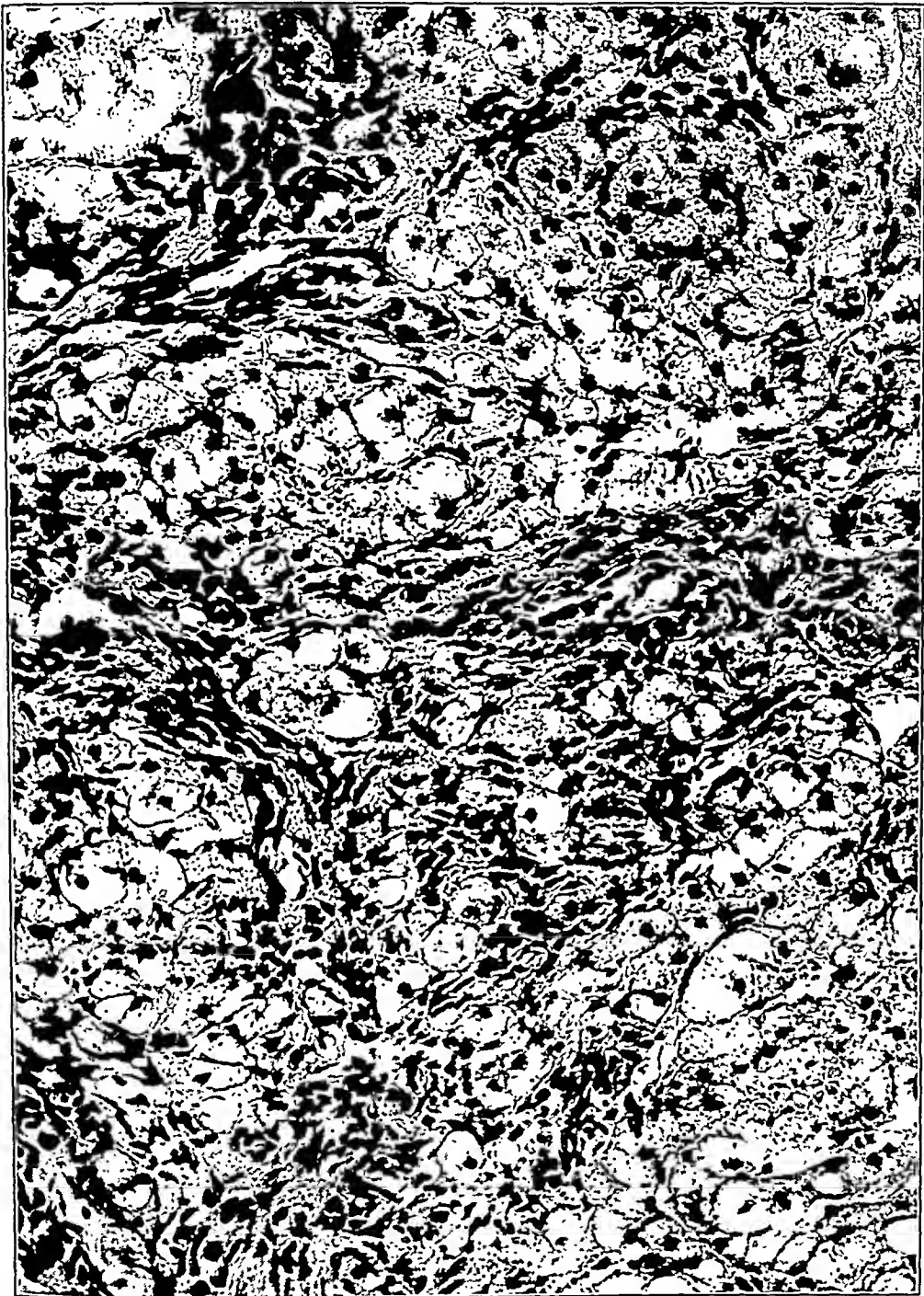


PLATE 23

FIG. 6. Liver of Dog DA showing details of fibroblastic proliferation in liver shown also in Fig. 5. Fat not prominent in cells, but the cytoplasm in many cells is lumpy and hyaline in appearance. Some rounded masses resemble "colloid" bodies. Dark, irregular homogeneous masses between strands of connective tissue are atrophied hyaline liver cells. Hematoxylin-eosin stain. $\times 300$.





PATHOLOGICAL CHANGES IN THE PLACENTA ASSOCIATED WITH ERYTHROBLASTOSIS OF THE FETUS *

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One of the earliest writers to recognize the importance of congenital edema of the fetus was Ballantyne.¹ He collected 70 cases, including those that had appeared in the literature and those that had come to his own personal notice. While the edema noted in many of these infants seemed to be due to obvious congenital defects, there were a few cases that showed no obvious etiological factor. It is this latter group that we have classified as part of the general syndrome of erythroblastosis, and it is in this group also that first mention is made of the gross placental changes in fetal dropsy.

Schridde,² first recognizing the underlying pathological changes associated with congenital hydrops, merely mentioned the placentas as showing edema both grossly and microscopically. In the same year Sitzenfrey³ noted both edema and hyperplasia of the stroma of the villi. Nyhoff⁴ stated that the villi were necrotic. Esch,⁵ Eichelbaum,⁶ Weiner,⁷ and Kovács⁸ also noted edematous changes in the villi. Goormaghtigh⁹ felt that there was erythroblastic proliferation and infiltration of the stroma.

Diamond, Blackfan, and Baty¹⁰ in an extremely comprehensive article on erythroblastosis have given a complete survey of the literature. In many of their references the authors have taken passing notice of the placental changes. This is especially true where the striking gross changes associated with congenital hydrops occur. In the same paper there appears a short microscopic survey of the pathological changes in the placenta.** For the first time mention is made of the apparent immaturity of the placenta. This feature has been similarly emphasized by Clifford and Hertig.¹¹

At no time, however, has a systematic examination of the placentas associated with both congenital hydrops and icterus gravis been recorded. It is the object of this investigation to record the histopathological changes in the placentas of infants

* Received for publication July 30, 1937.

** Description of the placenta contributed by Hertig.

suffering from erythroblastosis. For this purpose erythroblastosis has been divided into two subgroups — congenital hydrops and icterus gravis. The diagnosis of erythroblastosis has been made on finding, either clinically or at postmortem examination, an enlarged liver and spleen associated with an abnormal number of circulating nucleated red cells and extramedullary erythropoiesis. The term congenital hydrops has been used to designate the additional finding of anasarca, with or without icterus. The infants showing the additional finding of icterus without anasarca were classified under the term icterus gravis (Hellman and Hertig¹²). The majority of the cases herein presented occurred in the Boston Lying-in Hospital between the years 1931 and 1937. A few cases, however, were submitted to the pathological laboratory of this hospital for confirmation of diagnosis. These have been included with the permission of the attending physicians. The mothers of all the infants had negative blood tests for syphilis.* In addition, the liver, spleen and placenta of all infants in the hydrops group were stained for spirochetes by the Levaditi method. In no instance were any spirochetes found. For the sake of brevity only 1 case in each of the two groups will be presented in detail. The remainder will be presented in tabular form.

PRESENTATION OF CASES

Type 1. Congenital Hydrops

CASE 1. Mrs. H. B., No. 71756. The mother was a 29 year old, white, American-born para 5. She had had 2 normal children, 2 miscarriages and 1 stillbirth. Her prenatal course during this pregnancy was normal until 2 weeks prior to admission to the hospital when she began to have headaches. She developed a rapidly progressive type of toxemia and was admitted to the hospital as an emergency case. On admission she had edema of the hands, face and extremities, a blood pressure of 150/90, and a large trace of albumin in the urine. The laboratory test for syphilis was negative. In spite of all therapy she became progressively worse. On March 24, 1937, when she was 8 weeks from term, a Braxton Hicks version was performed and a foot brought down. She was subsequently delivered of a 2880 gm. stillborn female infant with advanced generalized edema. The clinical diagnosis of erythroblastosis of the hydrops variety was confirmed at postmortem examination.

Description of Placenta

The placenta weighed 1260 gm. The membranes were complete but badly lacerated. The fetal surface was smooth, shiny,

* The Hinton test for syphilis was used throughout.

gray in color and translucent. The cord showed no gross pathological change other than edema. The maternal surface was intact, a pale yellow gray, deeply fissured and extremely friable (Fig. 1). There was no calcification of the decidua. The placenta cut with increased resistance and the cut surface was a pale yellow gray and firm in consistence. The villi could be easily teased out.

On microscopic section the villi were larger than normal. The syncytial cells were large, and the nuclei were regularly spaced, large and vesicular. There were frequent paranuclear clear spaces which had a tendency to depress the adjacent nuclei. These vacuoles contained neither stainable fat nor glycogen. There were few so-called nuclear knots. There was a partial persistence of Langhans' layer. The villi presented stroma of both the hyperplastic and the edematous types, but the former was by far the more common. The hyperplastic type of stroma was made up of small cells with well stained acidophilic cytoplasm and small dark nuclei. The edematous type of stroma had fewer cells and these were widely separated by clear spaces. These cells were large, had a basophilic or pale acidophilic cytoplasm, were multipolar and gave off long fibrils. The nuclei were large and vesicular with peripherally arranged chromatin. In the interstices were many large mononuclear cells with a granular acidophilic cytoplasm (Hofbauer cells) which was often vacuolated. These vacuoles contained fat. The vessels were diminished in number and had a tendency to be arranged peripherally. The endothelium of the vessels was made up of large cells whose nuclei projected into the lumens of the vessels. The capillaries and larger vessels were all filled with nucleated red cells. In the villi with hyperplastic stroma were many foci of intracapillary erythropoiesis which showed red cells in all stages of development (Fig. 4). There were a few areas of subsyncytial fibrinoid deposition. The decidual and chorionic plates showed no pathological changes.

Type 2. Icterus Gravis

CASE 2. Mrs. S. L., No. R.H. 2673. The mother was a 38 year old, white, American-born para 2. Her first child was normal. The prenatal course during this pregnancy was entirely normal. The laboratory test for syphilis, although not done during the prenatal period, was negative on a follow up visit. She was delivered normally at term on Dec. 10, 1932, of a 3360 gm. male infant. The child was covered with a golden yellow vernix and was quite cyanotic at

TABLE I

Congenital Hydrops

Case No.	Age	Parity	Weight of placenta	Weight of fetus	Size of villi	Syncytial degeneration	Persistence of Langhans' layer	Epithelial vacuolization	Hyperplastic stroma	Edematous stroma	Diminution of vessels	Immature endothelium	Nucleated red cells	Erythropoiesis	Calcification	Infarction
	yr.		gm.	gm.												
S.C. R.H.864	36	4	1230	4140	++	+	++	+	+++	+	++	+	++			
M.K. 2827	37	2	1160	2880	++	+	++	++	+++	++	++	+++	+++	+++		
O.B. R.H.2420	22	2	1150	3240	++	+	++	++	+++	+	+	++	+++	++		
A.G. 786	33	9	1140	2910	+++	++	++	++	+	+++	++	++	+++	++		
E.R. R.H.2616	32	4	1040	3840	+++	+	++	++	+++	++	++	++	+++	++		
R.C. 5279	40	11	800	2490	++	+	++	+	+++	++	++	++	+++	++		I
E.M. 2832	30	4	1220	3890	++	+	++	++	++	++	+	+	+++	++		
M.D. R.H.2777	25	2	865	3420	++	+	++	+	+	+		+	++			
E.C. 12938	37	4	990	2730	++	+	++	++	++	+	++	++	+++	++		

E.C. 13627	37	7	1275	3450	++	+	++	+	+++	+	++	++	++	++			
F.K. 20651	27	3	1790	2355	+++	+	++	+	+++	+	++	++	++	++	++		
H.B. 71756	29	5	1260	2880	+++	++	++	+	+++	+	++	++	++	++	++		
H.W. 14577	39	12	1300	3600	+++	+	++	+	+++	+	++	++	++	++	++		
G.B. 19067	23	3	1080	2160	+++	+	++	+	+++	+	++	++	++	++	++	+	I
M.*	27	3	1183	3750	+++	+	++	+	+++	+	++	++	++	++	++		
W.†	29	4			+++	+	++	+	+++	+	++	++	++	++	++		

*Case submitted by Dr. A. E. Steele, Lawrence Memorial Hospital, Medford, Mass.

†Case submitted by Dr. R. Durkee, Hartford Hospital, Hartford, Conn.

TABLE II

Icterus Gravis

Case No.	Age	Parity	Weight of placenta	Weight of fetus	Size of villi	Syncytial degeneration	Persistence of Langhans' layer	Epithelial vacuolization	Hyperplastic stroma	Edematous stroma	Diminution of vessels	Immature endothelium	Nucleated red cells	Erythropoiesis	Calcification	Infarction
E.N. 5435	yrs. 21	2	gm. 810	3090		+++							+			
S.L. R.H.2637	38	2	570	3360	+	+	++	++	++	+	+	+	++			
C.S. 11649	27	2	610	3680	+	+++							+			
D.B. 16449	39	5	650	3360	+	+		+	+		+	+	+			
G.F. 16827	28	3	460	3210	+	++		++	+	+	+	+	+			
A.F. 8844	23	3	735	3750	+	++		+	+			+	+			
E.N. 5435	27	4	540	3570	+	+		+	+			+	+			

birth. He never breathed well. Shortly after birth he developed petechiae in the scalp. The liver and spleen were enlarged to palpation. Examination of the blood showed 4,100,000 red cells and 14,000 nucleated cells, 64 per cent of which were red cells. The hemoglobin was 105 per cent. The clotting time was 30 minutes, the bleeding time normal. In spite of a transfusion of 30 cc. of mother's blood the child continued to fail. The nucleated red cell count rose to 80 per cent of the nucleated cells. Jaundice appeared on the 2nd day and the child died shortly thereafter.

Permission for a postmortem examination was not obtained.

Description of Placenta

The placenta weighed 570 gm. The membranes were complete but were stained a slight yellow. The chorionic plate was thin, smooth and glistening. The maternal surface was intact, showed normal fissuring, and there was no calcification. There were several small areas of ischemic necrosis of the villi. The placenta cut with the usual resistance and the cut surface was pale reddish yellow. It was not unusually friable. The cord showed no pathological changes.

On microscopic section the villi were slightly increased in size. The syncytial cells were slightly larger than usual and the nuclei were more evenly spaced. The nuclei were large and vesicular and there were many paranuclear clear spaces that had a tendency to depress the adjacent nuclei. The so-called nuclear knots were somewhat reduced in prominence. There was a partial retention of Langhans' layer. The epithelial layers showed little degenerative change. The cellularity of the stroma was increased but was mostly of the edematous variety and the hyperplastic type was not as evident as in the placenta of Type 1. The same large cells with multipolar processes were present. In the interstices were many Hofbauer cells, most of which showed cytoplasmic vacuoles. The vessels were somewhat reduced in number but their endothelium showed no pathological changes. There were no areas of erythropoiesis but all the vessels contained nucleated red cells. The chorionic and decidual cells showed no pathological changes.

DISCUSSION

Table I records the gross and microscopic pathological changes in 16 placentas from infants suffering from erythroblastosis of the hydropic variety. The infant mortality in this group was 100 per

cent and the diagnosis in each instance was confirmed by post-mortem examination. In considering these pathological changes only the outstanding deviations from normal have been recorded. In an effort to give some estimate of the magnitude of this deviation, the symbols +, ++, and +++ have been used. A blank space represents no deviation from the normal. These values were based on the opinion of one individual and in spite of the necessarily attendant error showed a remarkable consistency.

The pathological changes tabulated in Table I are also shown in the microphotographs (Figs. 2-5). The placentas were nearly double the normal weight and the fetal placental ratio was reduced from the normal of 6:1 to 3:1. Grossly the placentas presented a friable gray maternal surface which was quite distinctive. Microscopically the villi were increased in size, epithelial degeneration was reduced to a minimum, and there was an abnormal persistence of Langhans' layer, which should have entirely disappeared by the 5th month. Vacuolization of the epithelium was present in each instance. Definite changes in the stroma were also noted. There was a diminution in the number of vessels and the endothelial cells were large and immature. Nucleated red cells were always present and foci of erythropoiesis were noted in all but 3 of the placentas.

The 7 placentas recorded in Table II were from infants suffering from erythroblastosis of the icterus gravis variety. The presence of the disease in these infants was proved either at autopsy or by the clinical findings of an enlarged liver and spleen, jaundice, and a greatly increased number of circulating nucleated red cells. The placentas in this group were normal in weight but, as can be seen in Table II, they presented microscopic pathological changes varying only in degree from those seen in the preceding group.

It is felt that the changes here described constitute a pathological syndrome pathognomonic of erythroblastosis. The cause of these pathological changes is obscure. However, it is worth noting that the immature placenta, especially during the period of its greatest growth, shows epithelial, stromal and vascular changes reminiscent of those described above. However, erythropoiesis and nucleated red cells are never seen in the abundance in which they occur in erythroblastosis. It is of interest, too, that anaplastic proliferating epithelium from hydatidiform moles and cases of chorionepithelioma shows vacuolization similar to that seen in the

immature placenta and the erythroblastic placenta. The presence of the Hofbauer cells in the immature placenta and that of erythroblastosis is of undetermined significance.

The resemblance of these pathological changes to those described as occurring in the syphilitic placenta is striking. Grossly the placentas are similar except for the peculiar fatty surface attributed to the syphilitic placentas. Microscopically the villi in both types are enlarged. Here, however, the resemblance ends, for the pathological changes described as being due to erythroblastosis do not resemble the fibrosis, obliteration and round cell infiltration of villous vessels attributed to syphilis. Although syphilis probably does produce certain changes in the placenta, it is the consensus of current opinion that too often the presence of a positive maternal Wassermann has led to a diagnosis of syphilis of the placenta when the changes present were due primarily to immaturity. A more careful consideration of the autopsy and placental material in these cases would probably disclose a number of cases of erythroblastosis. It is certain that the postmortem examination of every still-born infant should include both gross and microscopic examination of the placenta.

SUMMARY AND CONCLUSIONS

1. Sixteen placentas from infants suffering from erythroblastosis of the hydropic variety and 7 from infants suffering from erythroblastosis of the icterus gravis variety are presented.

2. Pathological changes in the epithelium, stroma and vascular tree are described which constitute a pathological syndrome pathognomonic of erythroblastosis of the hydropic variety.

3. Similar, but less advanced pathological changes are present in placentas from infants suffering from erythroblastosis of the icterus gravis variety.

4. These pathological changes resemble to some extent those seen in immature placentas and in the epithelium from hydatidiform moles and cases of chorionepithelioma.

5. The resemblance of these pathological changes to those attributed to syphilis is discussed.

6. In our experience no other disease of the fetus produces a similar picture in the placenta.

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DESCRIPTION OF PLATES

PLATE 24

FIG. 1. S-37-292. Placenta of the hydropic variety showing great increase in size and gray, deeply fissured maternal surface.

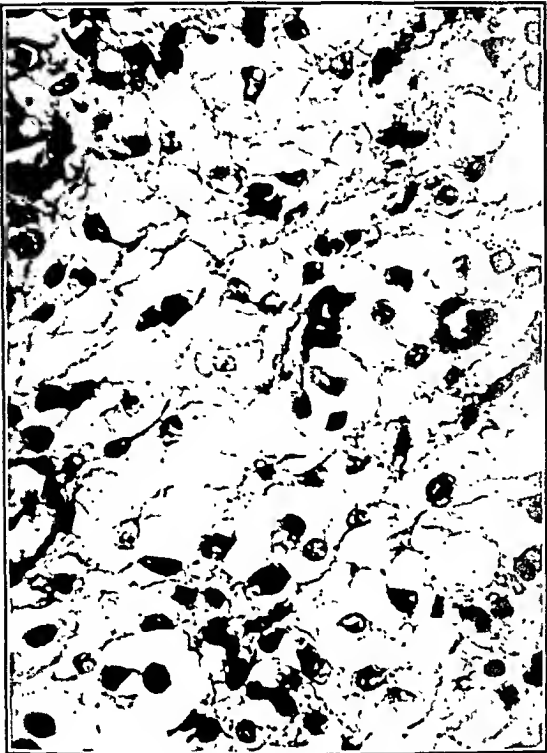


Hellman and Hertig

Pathological Changes in Placenta

PLATE 25

- FIG. 2. S-37-292. Villus from a placenta of the hydropic variety. Great enlargement of the villus is shown. The stroma is of both the hyperplastic and the edematous type. Hematoxylin-eosin stain. $\times 120$.
- FIG. 3. Same placenta showing edematous stroma with large multipolar stromal cells and vacuolated Hofbauer cells. Hematoxylin-eosin stain. $\times 420$.
- FIG. 4. Same placenta showing hyperplastic stroma with foci of erythropoiesis. Hematoxylin-eosin stain. $\times 420$.
- FIG. 5. Same placenta showing vacuolated immature syncytium and persistent Langhans' cells. Hematoxylin-eosin stain. $\times 420$.



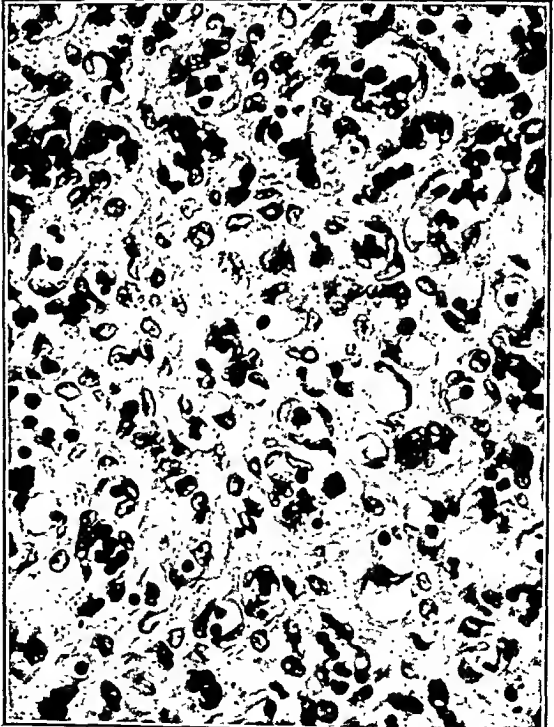
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CALCIFICATION OF THE AORTA, HEART AND KIDNEYS OF THE ALBINO RAT *

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Reports of the laying down of inorganic salts in the tissues of the ordinary laboratory rat are rare in the literature. Arteriosclerosis is thought not to occur spontaneously, although Hueper ¹ has reported that in the course of routine histological examination of 75 experimental rats 12 were found with extensive calcification of the pulmonary arteries. Other organs, such as the aorta and kidney, showed no such change. Duff ² makes the statement that he has found no independent information concerning the occurrence or frequency of spontaneous arteriosclerosis in rats, and with the exception of hypervitaminosis D, the rat does not respond to the experimental production of arteriosclerosis. Other observations on calcification of the kidneys of the rat have been reported. Polak ³ observed that kidney stones were found in nearly all young rats fed a complete diet with the addition of 2 to 3 per cent calcium carbonate. Eppright and Smith ⁴ have noted also that histological examination of rats receiving in addition to a basal diet a mineral supplement of calcium and phosphorus revealed extensive calcification of the kidneys.

During the past 6 months numerous instances of calcification have been observed in autopsies of rats in the course of life span studies in the laboratory of animal nutrition, Cornell University. The rats examined were from 3 experimental groups. Lesions were demonstrated in two ways: the aorta, heart and kidneys were X-rayed and then fixed for histological section. Tissues were fixed in Bouin's and Helly's solutions or in alcohol-formalin, sectioned at 10 μ and stained with hematoxylin and eosin or with silver nitrate.

The oldest of the animals belong to the last survivors of a group being studied for the effect of high (20 per cent) and low (8 per cent) protein diets on longevity. Six animals, males and females, ranging from 898 to 1160 days of age, were examined. There was

* This study was supported by the Rockefeller Foundation grant for research in longevity.

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no clear and definite evidence of calcification. Therefore, it may be concluded that age is not the most important determining factor in the production of arteriosclerosis.

The second group, which showed almost negative results, was a group of male animals ranging from 261 to 374 days of age, of which 48 were killed and 64 died. Of these, only 2 showed any evidence of arteriosclerotic lesions. The aortas of these animals showed small spots in the X-ray films. None showed calcification of the kidneys. In 8 instances, as will be mentioned later, there was evidence of beginning calcification in the formation of cartilage in the large blood vessels at the base of the heart.

The group of rats that showed a large percentage of calcification were representatives of an experimental group (male and female) designed to show the effect of slow growth on the life span. The general setup of this experiment was similar to that reported by McCay, Crowell and Maynard.⁶ Groups I and II represent controls, which received a basal diet adequate in all respects except for calory content, which was fed in addition. The basal diet was identical in composition and amount fed per rat as that in Group III. Group III *a*, *b*, *c* and *d* were allowed only enough of the basal diet for maintenance, and were thus retarded in growth. They were subjected to this procedure 200, 500, 700 and 850 days to date. As seen in Table I, the retarded animals show calcification to the greatest extent.

TABLE I
Frequency of Calcification

	Groups I and II (controls)	Groups III <i>a</i> , <i>b</i> , <i>c</i> and <i>d</i> (retarded growth)
Number of animals	8	12
Age in days	662-847	696-862
Calcification		
heart	4	10
aorta	4	12
kidneys	4	12

Examination of the X-rays revealed that in the aorta calcification occurred in two regions: the arch, and the abdominal region at the level of the renal arteries (Figs. 3 and 5). In the heart, calcification occurred most frequently in the large blood vessels close to the semilunar valves and only rarely in the ventricular region. In the kidneys there were found large accumulations in

the papillary region and often a distribution of fine particles over the entire medullary region (Fig. 1).

Histologically the findings in the aorta were similar to those seen in hypervitaminosis D. An accumulation of salts is deposited in the media without, apparently, any previous necrosis of the tissue or deposition of fat particles. The salts are deposited in fine particles along the fibers or in such masses as to make the media appear solid (Figs. 6 and 7). The only other visible change in the aortas is a loosening of the fibers with the appearance of spaces between them. This change, however, has been observed in all aortas of old animals and may be a normal indication of senility, the deposition of calcium being quite unrelated to it.

In the heart, calcification is often preceded by the formation of cartilage in the vessel wall (Fig. 9). This cartilage later becomes calcified. Additional evidence of this procedure is found among the younger rats previously mentioned where of the 48 killed 8 showed the beginning of cartilage formation in the vessels at the base of the heart (Fig. 8).

In the kidneys the calcium is deposited as concretions or stones (Figs. 2 and 4), or in the epithelium of the tubules (Fig. 2). The concretions are found in the collecting tubules near the renal pelvis, blocking the entrance, and often bulging into it. Only occasionally are stones found loose in the pelvis. Calcification of the epithelium of the renal tubules seems to indicate a previous necrosis of the cells.

No explanation can be given at the present time for the differences found in these groups. The nutrition may have been a factor since the diets were designed to permit the same ingestion of essential nutrients such as protein, vitamins and inorganic elements by each individual rat without regard to the body weight. In such experiments the retarded animals ingest more inorganic matter per unit of body weight than the normal growth controls. Further experiments will be necessary to test such a working hypothesis. These diets will be discussed in detail when this study is completed.

Another consideration which can not be disregarded is that of endocrine disbalance. The fact that the type of lesion found in the arteries is similar to that found after treatment with large doses of adrenalin or thyroxin is of interest. It has been shown

(Asdell and Crowell ⁶) that in retarded rats there is a definite cessation of sexual activity. This might well be due to a pituitary disturbance manifesting itself in other ways as well.

SUMMARY AND CONCLUSIONS

1. The aorta, heart and kidneys of the ordinary laboratory rat were examined by X-ray and by histological section for deposition of inorganic salts.
2. Calcification was found in all rats retarded in growth by decreased caloric intake.
3. Age was found not to be the primary determining factor.

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DESCRIPTION OF PLATE

PLATE 26

- FIG. 1. X-ray of kidneys, showing distribution of inorganic salts. Rat 21 III *b*, retarded 500 days. $\times 2$.
- FIG. 2. Section of kidney showing distribution of inorganic salts. Rat 25 III *a*, retarded 200 days. $\times 7$.
- FIG. 3. X-ray of aorta showing calcification in two regions. Rat 21 III *b*. $\times 2$.
- FIG. 4. Section of kidney after decalcification, showing a concretion. $\times 16$.
- FIG. 5. X-ray of aorta showing calcification along entire length. Rat 60 III *c*, retarded 700 days. Natural size.
- FIG. 6. Section of aorta at the base of the heart. Rat 21 III *b*. $\times 200$.
- FIG. 7. Section of aorta at arch. Rat 21 III *b*. $\times 200$.
- FIG. 8. Section through auricular region of heart showing cartilage formation. Rat k 494. $\times 100$.
- FIG. 9. Section through auricular region of heart. Rat 110 II. $\times 100$.

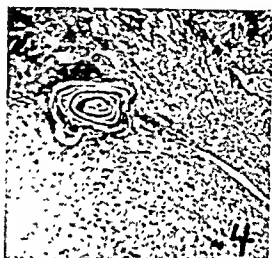


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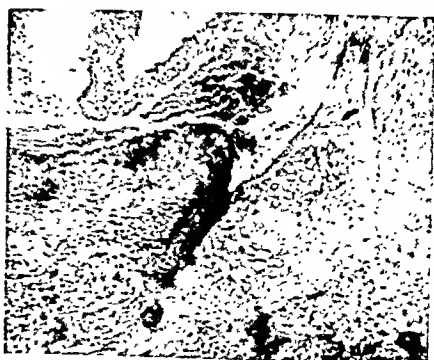


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NESIDIOBLASTOMA, THE ISLET TUMOR OF THE PANCREAS *

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This is a microscopic study of 9 "adenomas of the islets of Langerhans" removed surgically from 6 patients at the Presbyterian Hospital, New York City. It is a supplement to the report on these tumors published by Whipple and Frantz in 1935. The operations were performed for the relief of hypoglycemia with severe and long continued collateral symptoms of from 1 to 12 years duration. All patients recovered from the operation. In 5 patients the blood sugar rose and the collateral symptoms disappeared promptly. In Case 3, after removal of Tumor 3, there was no improvement. At a second operation, 1 month later, Tumor 4 was found and removed, together with 6 cm. of the tail of the pancreas, this time with prompt recovery.

The tumors were small, from 4 mm. to 2 cm. in diameter. In the tumors 2 cm. in diameter there was extensive fibrosis and calcification. In none of the tumors was there sufficient material for chemical or biological assay. The conclusions in this paper are based solely on histological staining, including specific staining of the cytoplasmic granules.

TUMOR PATTERNS

Structurally these tumors are nothing but gigantic islets of Langerhans, of which we have an excellent example in Tumor 1.

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Figure 1 shows how faithfully this tumor copies the structure of the normal islet. It reproduces the rich capillary network bordered by rows of columnar and cuboidal cells. As in normal islets, some of the capillaries have an endothelial lining, but many have none, the tumor cells seemingly being in direct contact with the blood. The tumor cells measure the same as cells of normal islets and are packed with fine granules which stain like the granules of the normal islet cells. As in normal islets, there is a minimum of fibrous connective tissue limited to a few strands along the capillaries and a delicate meshwork of argyrophil reticulin around the capillaries. Large areas of the tumor have no reticulin framework. Even with the highest magnifications and with a variety of stains, this tumor is indistinguishable from a normal islet except at the border where the adjoining acini are entangled and compressed in an incomplete fibrous capsule. The balance between tumor growth and blood supply is well maintained for all the cells appear healthy and there is no sign of necrosis or fatty degeneration.

Tumor 2, illustrated in Figure 2, is another gigantic islet of healthy cells. The figure shows the tendency of these tumors to exaggerate and repeat over and over some structural feature of a normal islet, in this instance the rosette arrangement of the cells around a capillary.

Figure 3 illustrates Tumors 5 and 6, removed at the same operation. This is an unusual pattern consisting of long ribbons of columnar cells with centrally placed nuclei. Each ribbon is a single row of cells lying between two capillaries. At many points no endothelial lining is visible and there is no fibrous connective tissue or argyrophil reticulin interposed between the tumor cells and the blood stream. The cells are packed with the specific islet cell granules. The cells of this tumor give an illusion of being abnormally large. By actual measurement they are quite uniformly the size of many normal islet cells.

Unusual as this pattern is, it is merely another instance of exaggeration and repetition of an ordinary islet figure. Short ribbons of this type occur in normal islets but the true prototype of the long ribbon is found in islet hypertrophy. MacCallum's picture of the ribbons in islet hypertrophy would serve as an excellent illustration of Tumors 5 and 6. Despite its resemblance to an embryonic structure, this ribbon pattern is not an embryonic or

undifferentiated form. Embryonic islets are more like the compact short ribbon type pictured in Figure 1.

As far as we know, no other islet tumor of the long ribbon type has been recorded, but the long ribbons of hypertrophy have been described by MacCallum, Cecil, and Weichselbaum and Stangl and regarded, mistakenly we believe, as an undifferentiated or regenerating form.

HYDROPIIC DEGENERATION

Hydropic degeneration was not observed in any of our tumors. In the absence of exact knowledge we refrain from speculation on the possible relation to excess production of insulin.

FIBROSIS

Pursuing our thought that the islet tumors are gigantic islets, we arrive at Tumors 3, 4, 5, 6, 8 and 9, all of which show more or less extensive fibrosis, hyaline degeneration and calcification. Here again the tumors are merely reproducing islet lesions on a grand scale; for non-tumoral islets are subject to precisely these changes — fibrosis, hyaline degeneration and calcification. The size of the tumors renders these lesions more impressive than when they occur in the tiny islets.

Fibrosis seems to be the common fate of these tumors. Of our 9 tumors, 6 present broad areas of fibrous connective tissue dotted with small groups of surviving tumor cells. In 1 of the 3 remaining tumors fibrosis is beginning in one sector. In most of the reports of islet tumors more or less extensive fibrosis has been recorded.

Figure 4 shows the fibrosis beginning in one sector of Tumor 1 as a thickening of the capillary wall studded with small blocks of collagen.

Figure 5 shows an advanced fibrosis, only a few tumor cells remaining; but these tumor cells are packed with the specific granules and they must have been active to judge by the prompt relief from the hypoglycemia after operative removal.

HYALINE DEGENERATION

In some of our fibrosed tumors much of the newly formed fibrous connective tissue has been converted into a clear glassy

substance which, in the negative outcome of amyloid and mucin reactions, we must be content to call hyalin. With Mallory's aniline blue collagen stain, or with its variants — Masson's trichrome and Heidenhain's azocarmine — the hyaline substance stains pale blue, much paler than the fibrous connective tissue. Many tumor cells contain similar pale blue patches in their cytoplasm. Studying these patches and the apparent conversion of entire cells to pale blue blocks, we are convinced that the tumor cells themselves undergo the hyaline as well as the fibrous connective tissue change, settling, in our own minds at least, the long-standing controversy as to whether the hyaline metamorphosis is restricted to the collagen or to the cytoplasm. It affects both.

This hyaline metamorphosis of the tumor cells has nothing to do with Bloom's D cells which stain with aniline blue.

CALCIFICATION

Three of our tumors are extensively calcified, which is not surprising considering the extent of the hyaline degeneration. As Mallory observes, hyaline material calcifies readily everywhere in the body.

SPONTANEOUS CURE

On viewing the extensive destruction of tumor cells by fibrosis and hyaline metamorphosis, one surmises that this process might proceed to total obliteration of the tumor cells and a spontaneous cure (Bensley, O'Leary). Against this conclusion is the fact that tumors of several years duration and with extensive destruction of cells are still capable of producing hypoglycemia, as shown by the prompt relief of this condition after their removal.

The situation reminds one of chronic tuberculosis where, despite extensive healing by fibrosis and calcification, the healing process never quite overtakes the advance of the tuberculosis. Among islet tumors there is no authentic instance of spontaneous cure.

NUCLEI

A word should be said about nuclei. Those pioneers in the study of islet cells, Lane and Bensley, described characteristic features of the nuclei of A and B cells and acinus cells, and their descriptions have been copied from one writer to another ever since with-

out adequate criticism. With the acid fuchsin-methyl green stain it is a simple matter to bring A cells, B cells and acinus cells into the same field for comparison. After a careful study of human and animal pancreas, and of the tumors, all fixed promptly in Zenker's or Bouin's fluid and properly stained, my conclusion is that there is nothing characteristic about the nuclei which distinguishes one of these cells from the other. When put to the practical test of diagnosis these meticulous nuclear distinctions break down, as they did with one experienced cytologist and student of the pancreas (O'Leary), who studied the 5 St. Louis tumors under the most favorable conditions — immediate fixation and expert staining — and concluded that "these characteristics are hardly sufficient to distinguish one type of cell from another." We agree with him.

SPECIFIC GRANULES

Islet cells and the cells of the islet tumors differ from most cells in the body by being packed with fine granules. These are probably secretion granules (O'Leary). They are not artefacts for they are visible in fresh islets (Laguesse, Bensley, Covell, O'Leary). Laguesse stained these granules with safranin; Lane with gentian violet and orange G; Martin with ethyl violet and orange G; Bowie with ethyl violet and Biebrich scarlet; and Bensley with a variety of methods, including acid fuchsin and methyl green. These stains were devised for the pancreas in the lower animals. In our hands, when applied to human pancreas and human tumors, these stains with one exception proved to be exasperatingly capricious. The exception was Bensley's acid fuchsin-methyl green, which we found to be simple, accurate and constant in all kinds of islets, normal and pathological, and in islet tumors.

If normal pancreas is fixed in Zenker's fluid and paraffin sections are stained with acid fuchsin and differentiated in methyl green, the acinus cells are green with green nuclei, the zymogen granules red, and the basal filaments and mitochondria red. In contrast with the green acinus cells the islet cells are packed with fine red granules. With a slight modification of the technique the granules of Lane's A cells hold the red, while the granules of the B cells turn purple. The tumor cells react to this stain exactly like islet cells. In most of the tumor cells the granules take the

purple color of B cells with here and there a red A cell. It should be noted that to get good differentiation of islet cells, human pancreas requires stronger and longer staining than the pancreas in the lower animals. In the tumors we have not found Bensley's granule-free C cells and we agree with O'Leary in failing to find any of Bloom's D cells that stain with aniline blue. D cells are supposedly brought out best by fixation in Helly's fluid (Zenker-formol), in which some of our tumor tissue was fixed.

ORIGIN OF THE TUMORS

In the pancreas, the duct epithelium is the source of all growth and repair (Bensley, Norbert, Grauer). In the embryo, epithelial buds from the duodenum grow toward the spleen as branching pancreatic ducts. This duct epithelium is totipotent, as Bensley calls it, for at one point it differentiates into acinus cells, at another point into islet cells, and at still other points it pushes forward as branching ducts. The duct epithelium retains this totipotency throughout life, as shown by the remarkable instances of regeneration of the pancreas from the ducts, reproducing the pancreatic structure complete, with acini, islets and ducts, amounting in several instances to regeneration of the entire pancreas of the adult rabbit (Grauer). Once differentiated out of the duct epithelium the islets grow by proliferation of their own cells (Bensley).

Curiously enough a stimulus that calls forth the duct-building and islet-building potency of the pancreas, while leaving the acinus-building potency in abeyance, is known. After ligation of the ducts both acini and islets degenerate and disappear, or nearly disappear (Bensley). If ligation is continued the islets regenerate from the ducts but the acini do not. If, on the other hand, the ligation is removed and free drainage of the duct system reestablished, the acini regenerate as well (Bensley, Harvey, Grauer).

The islet tumors may be regarded as a reaction of the duct epithelium to a stimulus that has called forth its duct-building and islet-building potencies, leaving the acinus-building potency in abeyance. Figure 6 from Tumor 3 shows the process in full swing. Throughout the tumor, ducts are so numerous that most of them must be accepted as newly formed. In the center of the figure a duct is seen, easily recognizable by the terminal bars or

“Schlussleisten” which fill the chinks between the epithelium. The epithelium of the duct is continuous with a group of tumor cells, as if the tumor cells were differentiating out of the duct epithelium.

Similar abundance of ducts and continuity of duct epithelium with tumor cells is found in every one of our tumors. O’Leary observed similar figures in 4 out of 5 of the St. Louis tumors, and interprets them in the same way. O’Leary observes justly that the mitotic figures in some of the tumors show that once differentiated out of the duct epithelium the tumor cells possess the power of independent proliferation.

In the normal pancreas such continuity of duct epithelium and islet cells is a matter of common observation (Laguesse, Bensley, and others). It is even asserted, on the evidence of serial sections, that the islets of the adult pancreas never lose their original continuity with the duct epithelium. The formation of the islet tumors, then, is merely an exaggeration of a normal procedure — differentiation of islet cells out of duct epithelium and subsequent independent growth.

Concerning mitoses and infiltration, we quote from Whipple and Frantz: “We have classified these eight tumors as adenomata, for the present at least, and only in the fifth, sixth, and eighth have we seen any evidence of what might be considered an infiltrating tendency. Marked variation in the size and shape of cells, mitotic figures in any appreciable number and blood vessel invasion are nowhere present.”

NESIDIOBLASTOMA

There is need for a short and accurate name for these tumors. Adenoma of the islets of Langerhans is long and cumbersome. Adenoma itself is vague, for we have already two kinds of adenoma, the benign epithelial tumor and lymphadenoma, quite different from each other. To add still another adenoma, an endocrine variety, merely adds to the confusion. We have followed current custom of suffixing “oma” to the Greek name of the cells of origin of the tumor. Selecting *νησίδιον* as the Greek word for islet, the cells that differentiate out of the duct epithelium to build islets may be called nesidioblasts — islet builders. When these islet builders, or nesidioblasts, form tumors, the tumor is a nesidioblastoma. The name has another application. In contrast with

the concentration of excess islet tissue in a tumor there is some evidence pointing to a diffuse or disseminated proliferation of islet cells as a possible cause of hypoglycemia. Such a diffuse proliferation of nesidioblasts would be a nesidioblastosis.

SUMMARY

Microscopically the chief feature of most of the tumors is their exact duplication of the pattern of normal islets. They also resemble islet hypertrophies in their tendency to exaggerate some features of the normal islet pattern. Just as the tumors duplicate the structure of normal and hypertrophied islets, so they are subject to the same pathological vicissitudes such as fibrosis, hyaline degeneration and calcification. The origin of the tumor cells is indicated by the abundance of figures showing the epithelial lining of the duct continuous with a group of tumor cells.

The origin of the name "nesidioblastoma" is explained.

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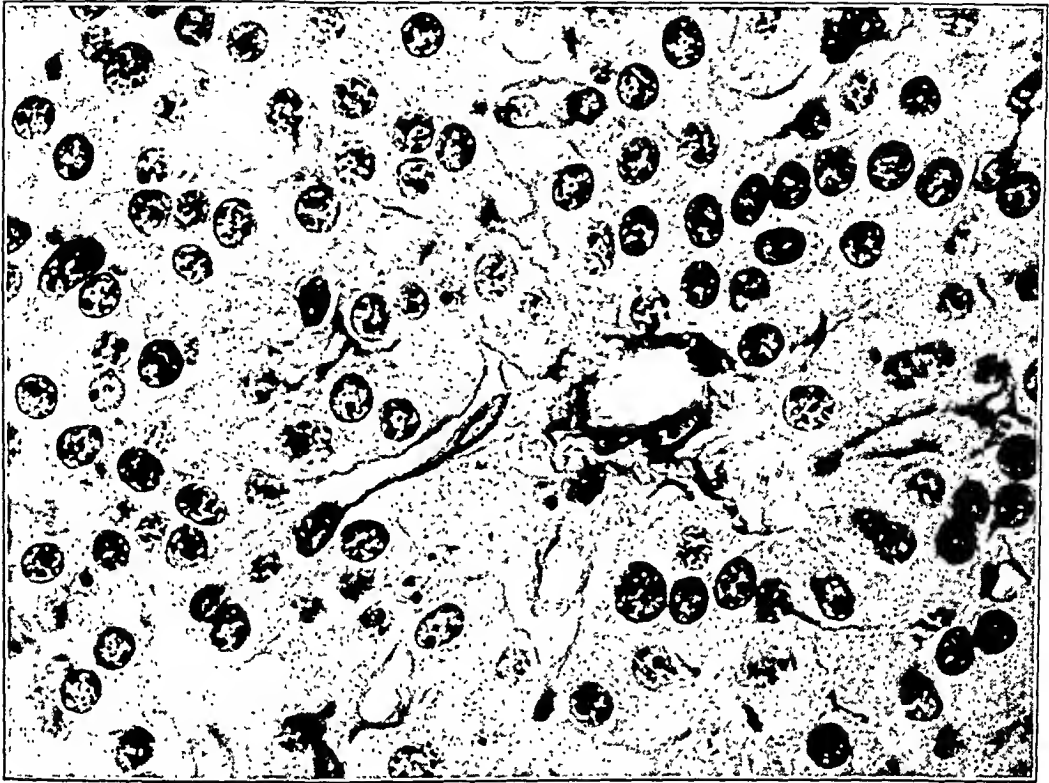
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DESCRIPTION OF PLATES

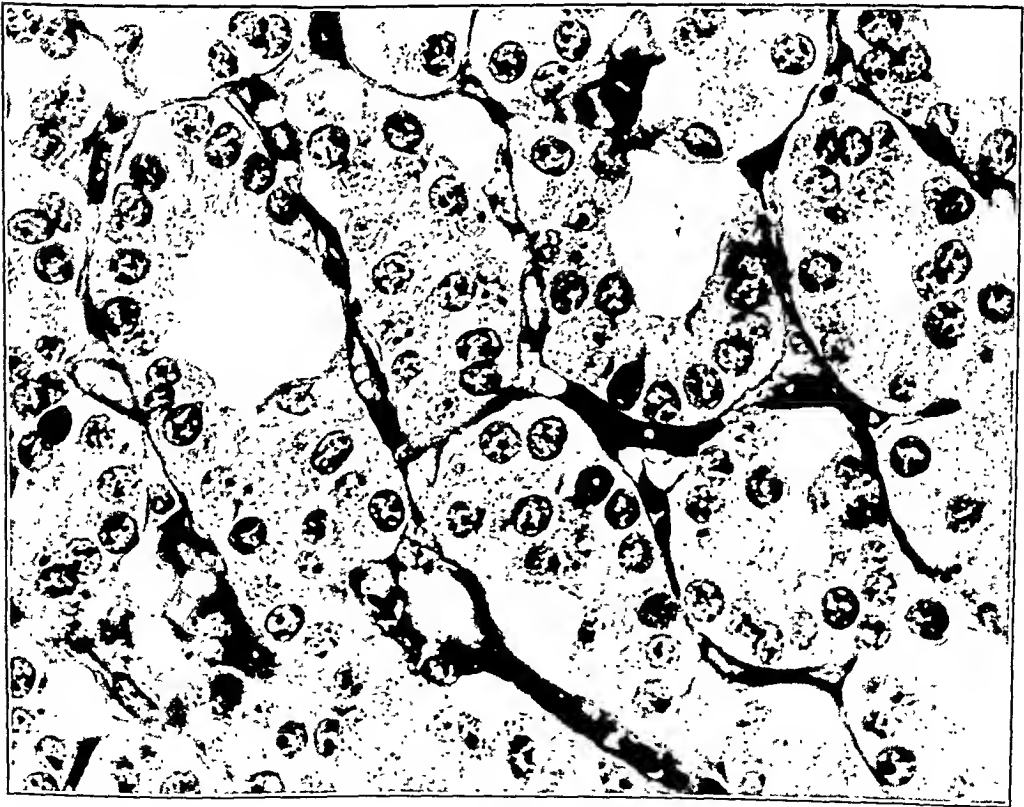
PLATE 27

FIG. 1. Tumor 1. The photomicrograph shows how the tumor faithfully reproduces the structure of the normal islet with its rich capillary network bordered by rows of columnar and cuboidal cells. Azocarmine stain. $\times 730$.

FIG. 2. Tumor 2. In this tumor there is a rosette arrangement of the cells around capillaries. This reproduces one feature of normal islets. Azocarmine stain. $\times 730$.



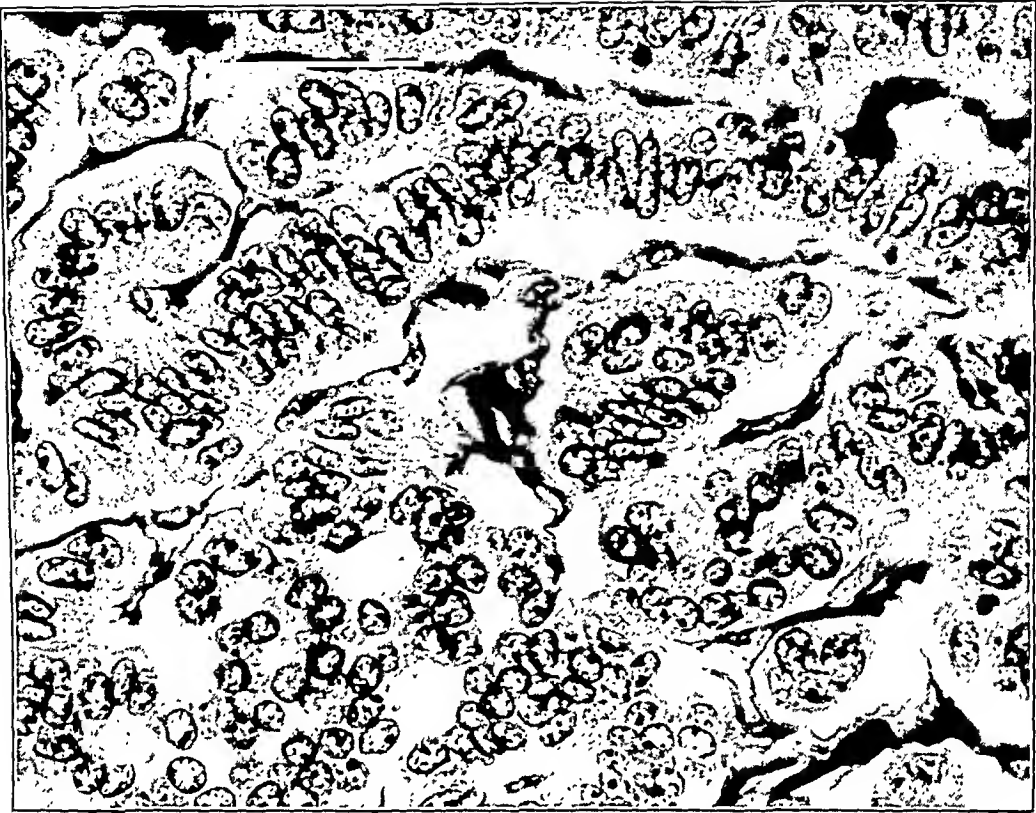
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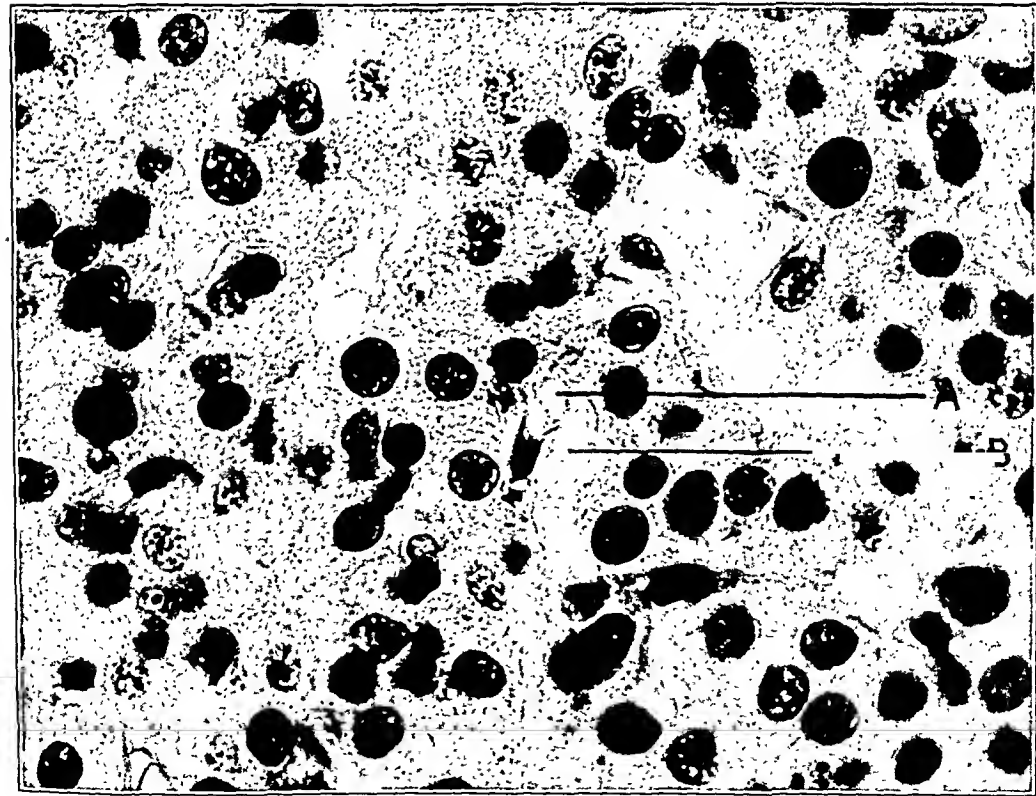
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PLATE 28

- FIG. 3. The photomicrograph shows the pattern of Tumors 5 and 6; long ribbons of columnar cells with centrally placed nuclei, each ribbon a single row of cells lying between capillaries. Azocarmine stain. $\times 730$.
- FIG. 4. This section of Tumor 4 shows fibrosis beginning as thickening of the capillary walls studded with small blocks of collagen. At A is shown the lumen of a capillary and at B a small block of collagen in its wall. Other collagen masses may be distinguished by the absence of granules. Masson's aniline blue-acid fuchsin-ponceau stain. $\times 730$.



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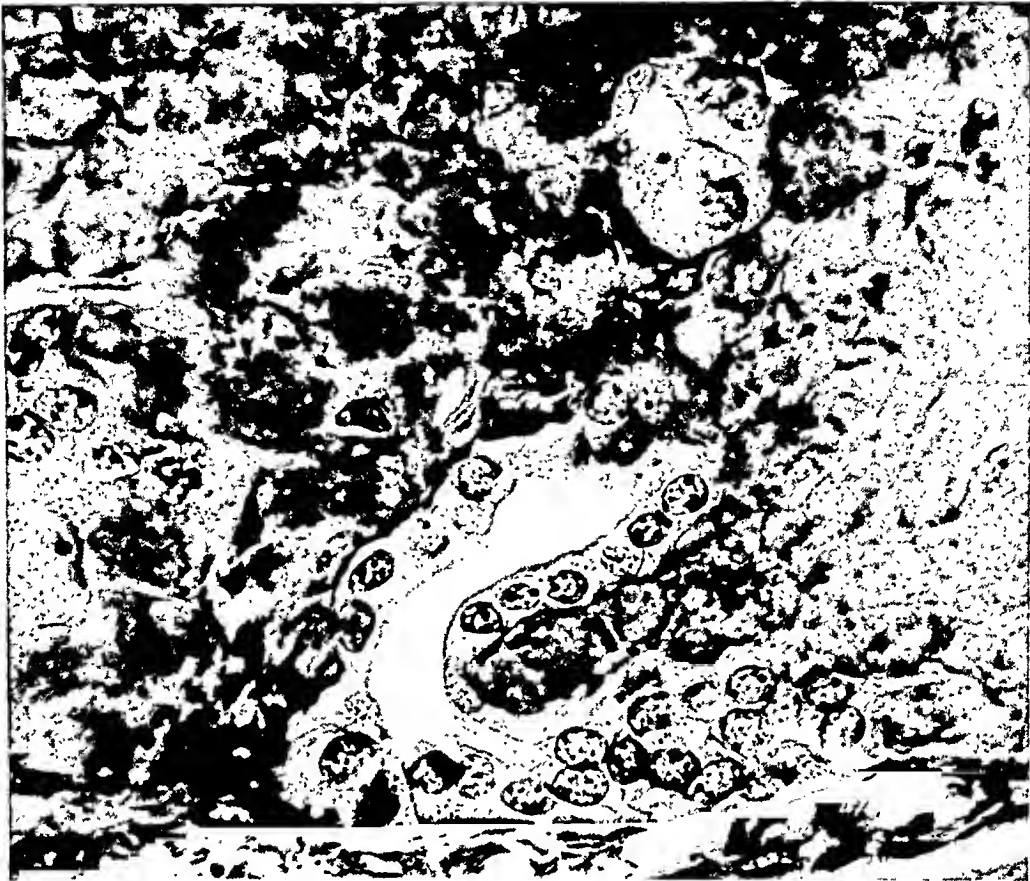


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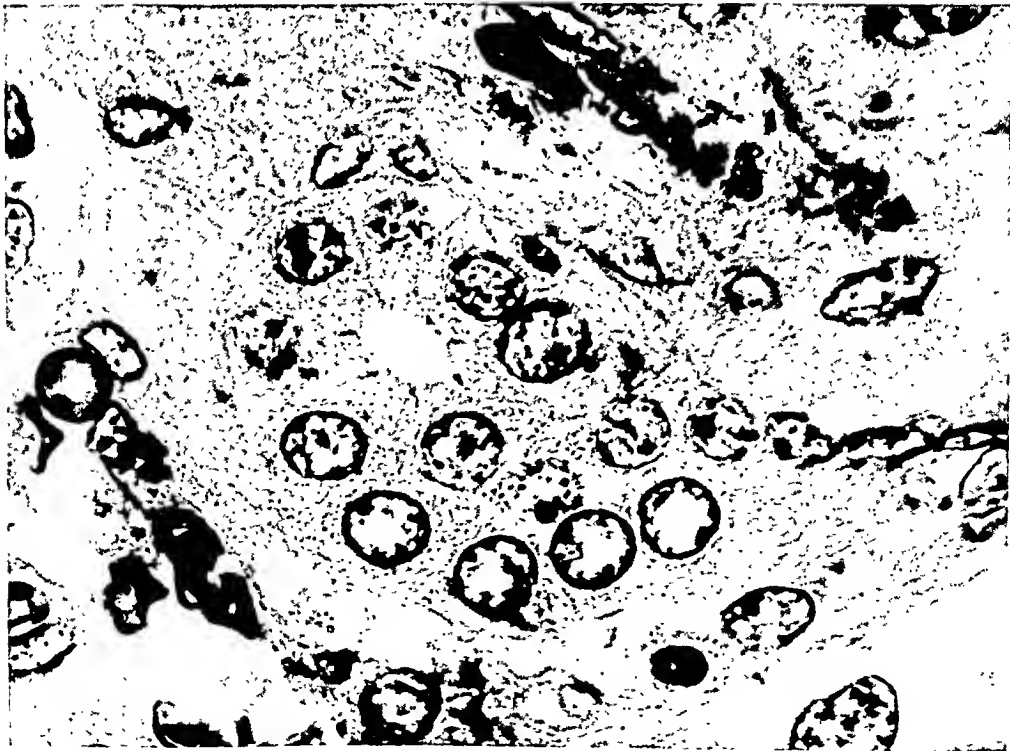
PLATE 29

FIG. 5. Tumor 3. An example of advanced fibrosis; only a few tumor cells remain but they are apparently active, judging by the presence of granules and the prompt relief of hypoglycemia after their surgical removal. Azocarmine stain. $\times 730$.

FIG. 6. Tumor 3. This shows the epithelium of the duct in continuity with a group of tumor cells, as though the tumor cells were differentiating out of the duct epithelium. The Schlussleisten are shown as black dashes between the cells lining the lumen and radiating from it. Masson's aniline blue-acid fuchsin-ponceau stain. $\times 1600$.



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DEVELOPMENTAL DEFECTS AT THE FORAMEN OVALE *

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Many individual instances of patent foramen ovale are already on record in the literature. These cases, however, do not seem to have been studied as a group, either with a view to differentiating the types of malformation encountered in this location, or ascertaining for the different kinds of defects the possible range of their variation in extent. A long-standing interest in the normal and defective development of the heart has, through the generous cooperation of colleagues, brought to me for study more material of this type than one person would ordinarily encounter. Recently a leave of absence afforded the further opportunity of studying the specimens accumulated in a group of pathological institutes with records covering a total of over 500,000 autopsies. Naturally not all the congenitally defective hearts from these autopsies had been preserved, but the extensiveness and variety of the material available was exceptional. Using drawings made directly from my own or museum specimens as a basis, and supplementing this material from a study of the literature, I have attempted to assemble a brief, but freely illustrated, survey of the defects that may be encountered at the foramen ovale. Being not a clinician but an embryologist, I have approached the subject from a morphological standpoint. It is hoped, however, that the material may prove a useful foundation for those interested in attacking the clinical problems associated with such defects.

LITERATURE

Publications dealing with failure of the foramen ovale to close have been appearing for more than three centuries. Many of the papers are so old that their viewpoint has become almost unintelligible to us of today. Botalli in 1565, for example, seized on cases exhibiting an open foramen ovale as offering an improvement on Galen's idea that the blood entered the left side of the heart from the right by way of spaces between the trabeculae of the interventricular septum (Dalton, 1884, p. 137). The weight

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of Botalli's name behind this erroneous conception delayed for many years the acceptance of Servetus' contention that the passage of blood from right to left "does not take place through the median wall of the heart as commonly believed; but, by a grand defice, the refined blood is driven from the right ventricle of the heart, in a long course through the lungs." The language in which Servetus elaborated his ideas well indicates the curious mixture of keen observation and dogma that pervaded the work of this period. "By the lungs it (the blood) is prepared, assuming a bright color. It is mingled with the inspired air and purged of its fuliginous matter by expiration and so at length the left ventricle of the heart attracts by its diastole the whole mixture, a suitable . . . material that . . . may become vital spirit." (Translation from Dalton, 1884, p. 115.)

Unfortunately the old papers are by no means the only ones in the literature that throw little light on the subject. Many comparatively recent articles are but superficial descriptions of isolated cases. An idea of the frequency with which papers based on 1 or 2 cases appear in the literature may be gathered from the fact that in 205 references cited by Poynter (1919) only 225 cases are involved. Many of these were merely clinical diagnoses of "open foramen ovale" with no confirmation by autopsy. Among the enormous number of papers on the subject disappointingly few contain both a good clinical history of the case and an adequate record of the autopsy findings.

Viewing the literature as a whole there seem to have been three factors primarily responsible for the often contradictory and unsatisfactory information it contains. First is the deep rooted tradition that the foramen ovale closes immediately following birth. Thus, in the absence of other findings accounting for death, an open foramen ovale in a young infant is frequently unjustly accused. This has led to much misapprehension as to both the frequency of occurrence, and the functional significance, of an unclosed foramen ovale during the neonatal period. There has long been ample evidence that the foramen ovale is not closed immediately after birth, but that its closure is a gradual process spreading over most of the first year (Aleksieyeff, 1901; Alvarenga, 1869; Elsässer, 1852; Hinze, 1893; Patten, 1930, 1931; Scammon and Norris, 1918). Familiarity with this fact would have eliminated

from the literature many papers describing as instances of "abnormal patency of the foramen ovale" conditions perfectly normal for the age at which they were observed. For example, a paper published comparatively recently in a well known medical journal is based on the heart of an infant that lived but 6 hours after birth. Death was attributed to an open foramen ovale and an unclosed ductus arteriosus!

A second cause of confusion commonly encountered in the literature is the failure to distinguish between conditions in which the foramen ovale is adequately covered by a valve which is not completely adherent to the septum, and conditions in which a structural defect of the valve or the septum makes it impossible for the foramen to be functionally closed. Incomplete adhesion of the valvula to the septum, with a resulting "probe-patency," is so common that it must be regarded as a variant of the normal rather than as an abnormality. The combined figures of ten different observers compiled from over 4000 autopsies in which this condition was an object of special attention show that probe-patency exists in one out of every four or five adult hearts (see Table I). As long as the valvula foraminis ovalis adequately overlaps the limbus fossae ovalis, probe-patency appears to be no functional handicap to an otherwise normal individual. The inclusion in the literature of a large number of cases where the "defect at the foramen ovale" was mere probe-patency has led to the impression that functionally significant defects in this region are much more common than is actually the case.

Still a third underlying difficulty in arriving at any clear interpretation of the significance of defects at the foramen ovale is one that seems inherent in the entire subject of congenital defects of the heart. There appears to have been a sort of collector's instinct obsessing contributors to this field. The more bizarre and complicated the case, the more interest it appears to arouse. From either the practical or the scientific standpoint this is unfortunate. The clinical picture especially is most confusing when several defects co-exist in the same heart. The only hope of arriving at any sound interpretation of such cases lies in better understanding of the developmental conditions responsible for, and the clinical manifestations of, uncomplicated cases of specific defects in which the major characteristics of the condition stand out unequivocally.

To attempt to give a systematic survey of all the articles in a field where such a large proportion of the material is either antiquated or uncritical would not be profitable. In the course of preparing this paper about 3000 references on congenital defects of the heart were culled. Some 300 of these purported to deal with an open foramen ovale. Even this burdensome list undoubtedly fails to constitute a complete bibliography, for the literature is scattered among journals dealing with clinical medicine, pathology, physiology, anatomy, embryology, and even general biology. It has, therefore, seemed wiser to dismiss the literature as a whole with the foregoing general comments and deal only with a relatively few selected references in connection with matters on which they were found helpful.

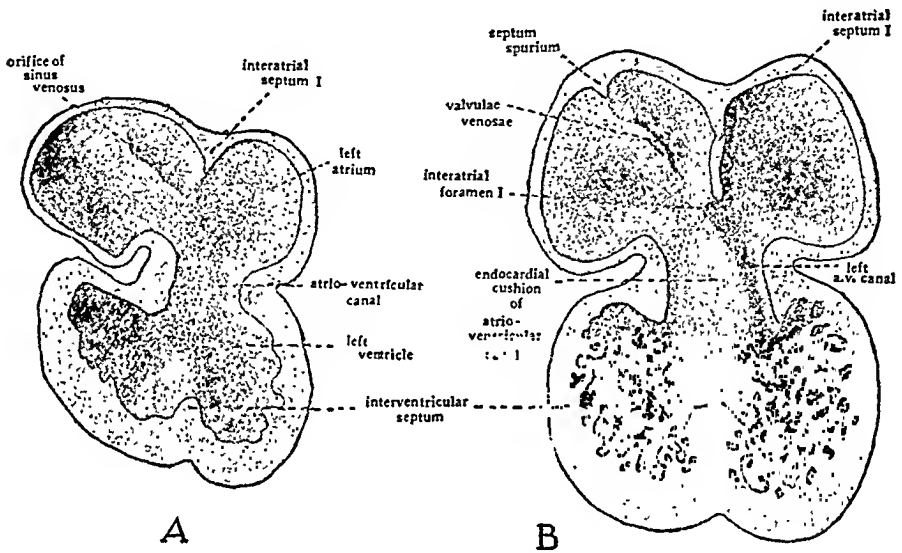
THE DEVELOPMENT OF THE INTERATRIAL SEPTAL SYSTEM

The growth processes leading toward the establishment of conditions as they appear in the heart of a newborn infant and the changes in the heart following birth are fairly well covered in the embryological literature (Born, 1889; Keibel and Mall, 1910; Mall, 1912; Odgers, 1935; Patten, Sommerfield and Paff, 1929; Tandler, 1912 and 1913; Waterston, 1918). Much of this information, however, is so widely scattered and so uncorrelated that it is not readily utilizable by those working in other fields. For this reason, and also for the sake of emphasizing certain points especially pertinent to an understanding of the defective conditions under discussion, the following brief summary of the normal prenatal and postnatal development of the interatrial septa is given.

In the separation of the primitive common atrium into right and left chambers two septa are directly involved. These, on the basis of their sequential appearance, are commonly called septum primum and septum secundum. The partitioning process starts in very young embryos, indications of the formation of septum primum being recognizable as early as the 5th week * of development. Starting as a crescentic ridge on the dorsocephalic part of the atrial well, septum primum grows toward the atrioventricular canal (Text-Figs. 1, A and 4, A).

* Ages as here given are approximate fertilization ages, for "menstrual age" add 14 days.

At about the same time that septum primum is making its appearance, the first indications of the impending division of the original common atrioventricular canal into a right and a left channel become evident. Two local thickenings, one dorsally, the other ventrally located, appear in the walls of the canal. These thickenings are the so-called endocardial cushions of the atrioventricular



TEXT-FIG. 1. Semischematic drawings of the interior of the heart to show the initial steps in its partitioning. (From Embryology, Patten, B. M., courtesy P. Blakiston's Son and Co.)

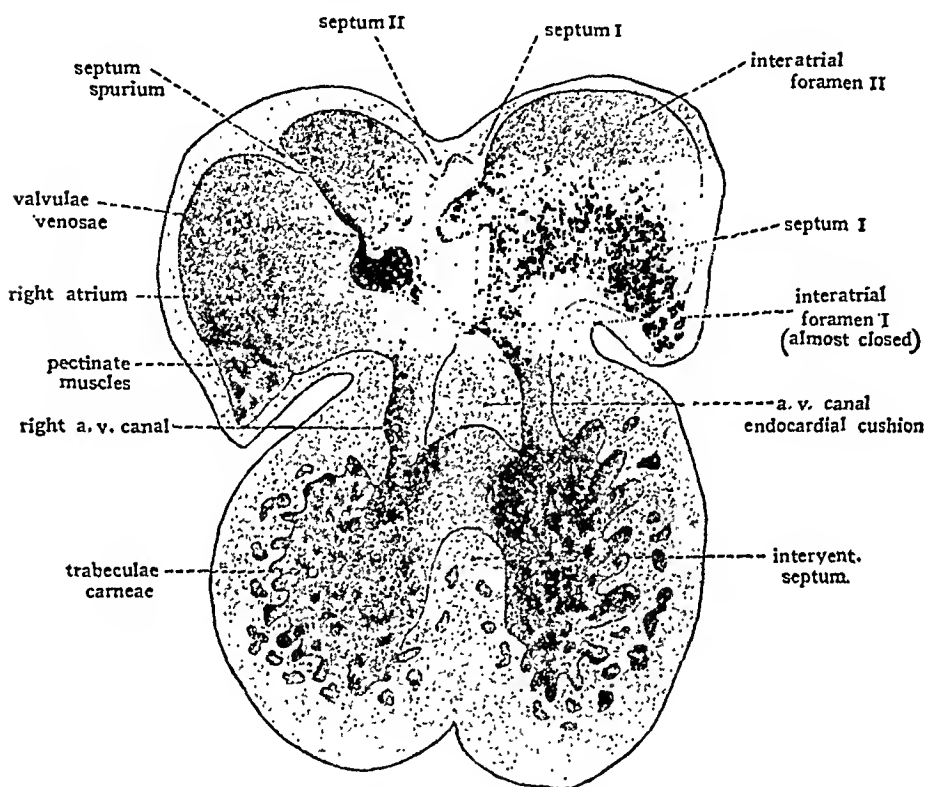
A. The cardiac septa are represented at the stage reached in human embryos early in the 5th week of development. Note especially the primary relations of interatrial septum primum. Based on original reconstructions of the heart of a 3.7 mm. pig embryo, and on Tandler's reconstructions of corresponding stages of the human heart.

B. The cardiac septa as they appear in human embryos of the 6th week. Note the restriction of interatrial foramen primum by the growth of interatrial septum primum. Based on original reconstructions of the heart of a 6 mm. pig embryo, on Born's reconstructions of the rabbit heart, and Tandler's reconstructions of corresponding stages of the human heart.

canal. Each cushion consists of a plastic mass of embryonal connective tissue, of the type characteristically appearing in the developing heart at points where septa will fuse, or where elaborate connective tissue structures such as the cardiac valves are destined to be moulded. During the 6th week of development the dorsal and ventral cushions are brought into contact with each other by their own growth and fuse to form a common mass dividing the

atrioventricular canal (*cf.* Text-Figs. 1 and 2, and Text-Fig. 4, C and D).

Between the concave margin of septum primum and the growing atrioventricular canal cushions is a progressively diminishing opening known as the interatrial foramen primum, or ostium primum



TEXT-FIG. 2. Semischematic drawing of the interior of the heart to show the start of interatrial septum secundum and the appearance of interatrial foramen secundum in septum primum. Based on original reconstructions of the heart of a 9.4 mm. pig embryo and on Tandler's reconstructions of the heart of human embryos of the 7th week. (From Embryology, Patten, B. M., courtesy P. Blakiston's Son & Co.)

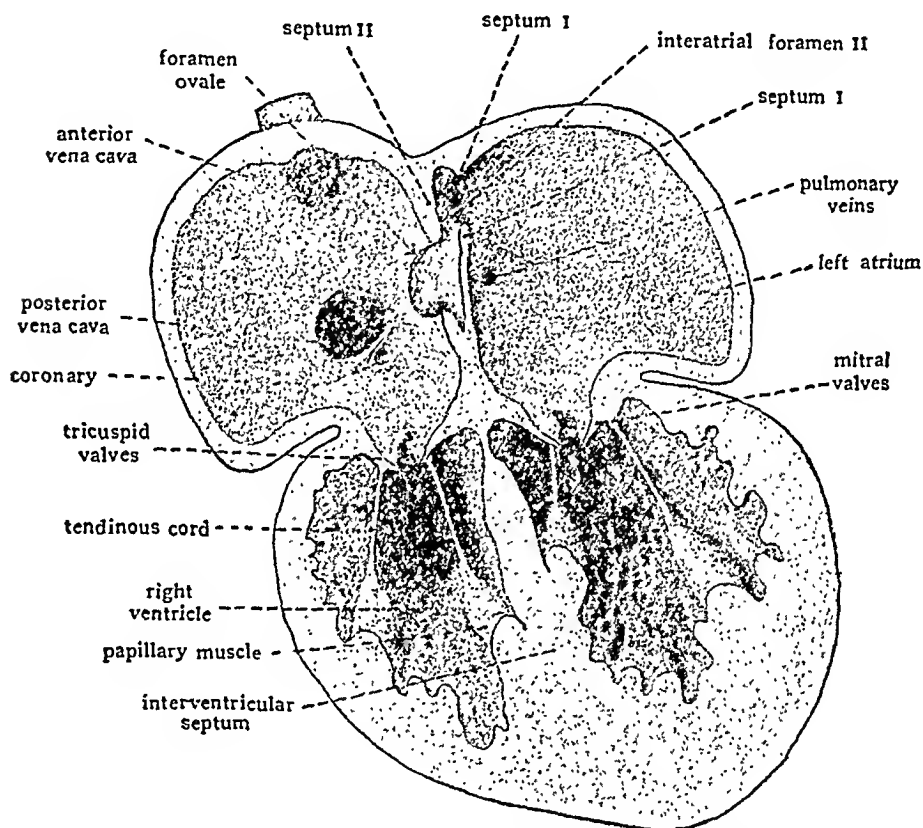
(Text-Fig. 1, B and 4, B). About when it seems as if the closure of the ostium primum would shut off the left atrium from the right (Text-Fig. 4, C) a secondary opening develops in septum primum near its origin from the dorsocephalic atrial wall. This new aperture first appears as multiple small perforations which soon coalesce to form a single opening known as the interatrial foramen secundum, or more briefly, as ostium secundum (Text-Fig. 2, and Text-Fig. 4, C and D).

The appearance of a second interatrial communication just as

the initial one is closing is of fundamental functional significance. In early embryonic life, when the lungs are as yet undeveloped, the left atrium lacks any considerable direct intake of its own. The constant presence of an interatrial communication makes it possible for the left atrium to receive without interruption a contribution from the blood entering the right atrium. More than the atrial part of the heart is involved in this matter of balanced atrial intakes for, as we have seen, the atrioventricular canal is divided by the 10 mm. stage, and at about the 15–17 mm. stage the inter-ventricular septum separates the right and left ventricles from each other. After these partitions are completed, if the atrial intakes were unbalanced the ventricular intakes would inevitably be correspondingly disturbed. That this is a matter of more than theoretical importance is clearly shown by the conspicuously defective development of the left side of the heart which is encountered when, as occasionally happens, abnormal development prematurely closes or markedly narrows the interatrial communication of the fetal heart. (A case of this type is presented later in this paper, see Figs. 17, 18 and 19.)

About the time the secondary interatrial opening is formed in septum primum, another septum begins to develop. The second septum is usually first readily recognized in embryos of about 12 mm. (end of 6th week), although occasionally its beginnings may be made out somewhat earlier. Like septum primum, septum secundum is crescentic in shape, but the open part of the crescent is directed more dorsally — toward the sinus inlet rather than toward the atrioventricular canal as was the case with septum primum. In reconstructions of the developing heart septum secundum can be seen lying just to the right of septum primum (Text-Fig. 2). Its cephalodorsal limb extends along the dorsal wall of the atrium with its tip lying in close association with the left valve of the sinus venosus. The ventrocaudal limb of septum II extends along the ventral walls of the atrium, sweeps caudally and merges with the atrioventricular canal cushion just to the right of the place where septum primum fuses with the canal cushion to obliterate the primary interatrial foramen (ostium I) (Text-Fig. 2 and Text-Fig. 4, D). The extreme tip of the ventrocaudal limb of the septum extends to meet the tip of the cephalodorsal limb at the base of the left valve of the sinus venosus (Text-Fig. 2).

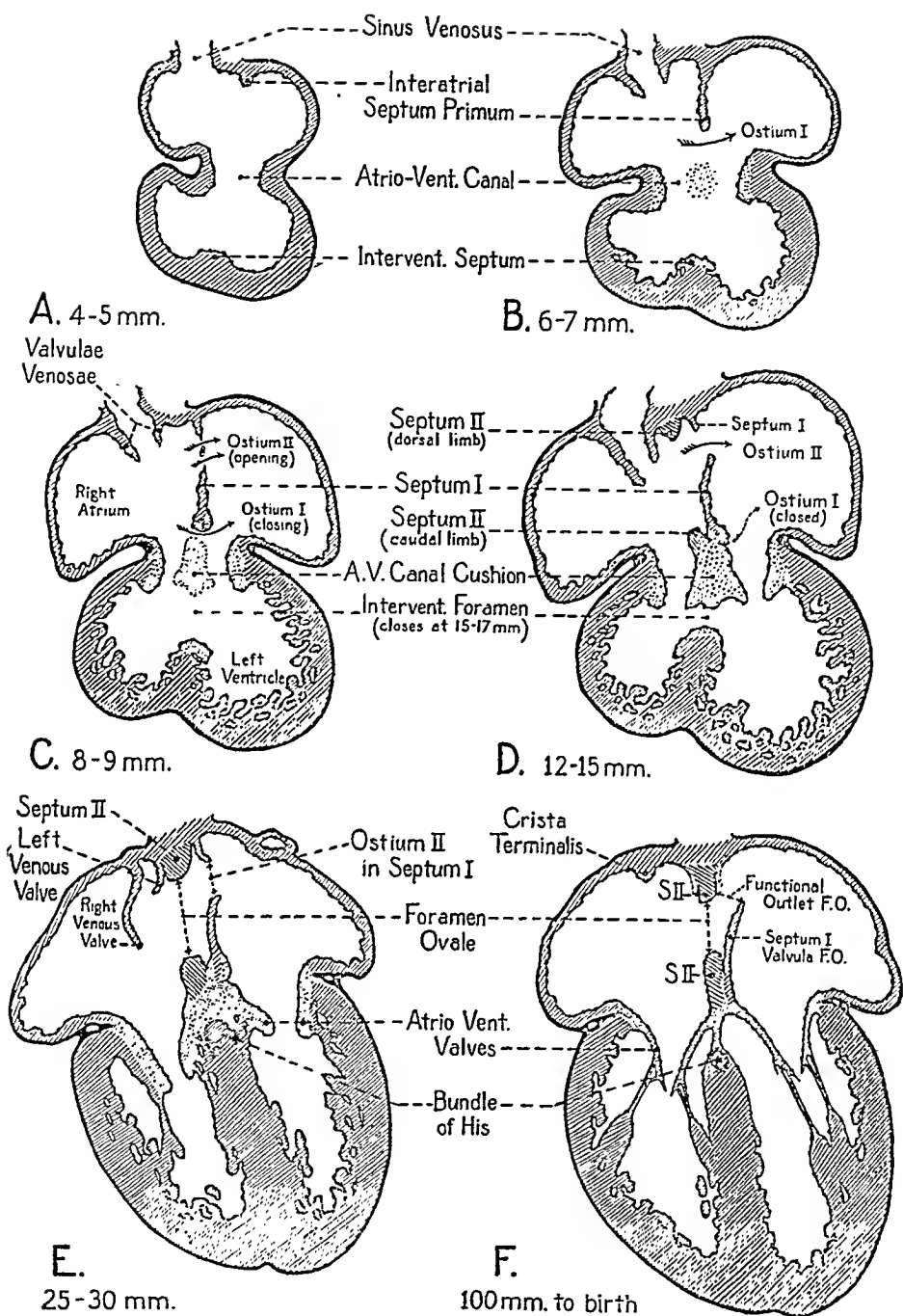
As septum secundum grows, its concave margin for a time cuts progressively farther into the atrial lumen; but septum I is not destined to become a complete partition. Its extension gradually ceases, leaving a characteristic oval aperture which is the foramen ovale (Text-Fig. 3 and Text-Fig. 4, E, F). The margin of septum



TEXT-FIG. 3. Schematic drawing to show the interrelations of septum primum and septum secundum during the latter part of fetal life. Note especially the way in which the lower part of septum primum is situated so it acts as a one-way valve at the oval foramen in septum secundum. (From Embryology, Patten, B. M., courtesy P. Blakiston's Son & Co.)

secundum thus constitutes what in adult anatomy is called the limbus or annulus fossae ovalis.

The relations of septum primum to the oval foramen persisting in septum secundum are of vital importance. The secondary opening in septum primum is formed so near the cephalic wall of the atrium that the unresorbed lower part of septum primum lies as a loose flap covering, on its left atrial side, the oval opening in septum secundum (Text-Fig. 3 and Text-Fig. 4, F). In this position it acts as one-way valve, permitting the filling of the left atrium



TEXT-FIG. 4. Sectional plans of the embryonic heart in the frontal plane, showing extent of growth of the various cardiac septa at several stages of development. These diagrams give specifically for the human embryo a more precise picture of the rate of progress of partitioning than do the schematic drawings of Text-Figs. 1-3.

Stippled areas in the diagrams indicate the distribution of endocardial connective tissue, muscle is shown in diagonal hatching, and the epicardium in solid black. The lightly stippled areas in the atrioventricular canal in B and C indicate the location of the dorsal and ventral endocardial cushions of the atrioventricular canal before they have grown sufficiently to fuse with each other in the plane of the diagram.

from the right but effectively shutting off return flow. In the fully formed fetal heart this flap is commonly known as the *valvula foraminis ovalis* rather than by its embryological name, *septum primum*.

Thus during intrauterine life we find a succession of three morphologically distinct interatrial communications, the first below *septum primum*, the second in *septum primum*, and the final one in *septum secundum*. This permits the left atrium, throughout fetal life, to receive a contribution of blood from the right atrium by a transseptal flow which compensates for the relatively small amount of blood entering the left atrium by way of the pulmonary circuit, and maintains an approximate balance of intake into the right and left sides of the heart. The amount of this compensatory interatrial flow changes in relative volume at different ages. Early in development, before the lungs have been formed, the flow from the right atrium through the interatrial ostium primum constitutes the entire intake of the left atrium. After ostium primum is closed and while the lungs are but little developed, flow through the interatrial ostium secundum must still be the major part of the blood entering the left atrium. During the latter part of fetal life the foramen ovale in *septum secundum* becomes the transseptal route. As the pulmonary circulation increases in volume, a progressively smaller proportion of the left atrial intake appears to come by way of the foramen ovale and a progressively larger amount from the vessels of the growing lungs. At the time of birth, on the basis of orifice measurements, somewhat more than half the blood entering the left side of the heart appears to come from the lungs and less than half from the right atrium by way of the foramen ovale (Patten, 1930).

The progressively diminishing transatrial flow and the progressive increase in the volume of the pulmonary circulation during the latter part of fetal life seem to have been largely overlooked. Unfortunately, no one has as yet solved the difficult problem of obtaining from living embryos pressure and volume determinations such as would permit a quantitatively accurate evaluation of the situation. But, if we may judge anything from the size of the vessels concerned, the volume of the pulmonary circulation of a term fetus is far from the negligible quantity commonly assumed. On the contrary, it is probably already sufficient to take care of

gaseous interchange as soon as the lungs are ventilated, for the pulmonary arteries of a term fetus are of approximately the same size as its umbilical arteries, and the total cross sectional area of the pulmonary veins is approximately equivalent to the cross section of the umbilical vein (Patten and Toulmin, 1930). If we recognize the fact that pulmonary vessels as large as the umbilical vessels can carry a volume of blood sufficient for gaseous interchange we are at once relieved of the necessity of postulating the traditional revolutionary changes in circulation at the moment of birth. Postnatal circulatory changes can then be interpreted on the basis of gradual readjustments which are more in harmony with what we know of other processes of change in living organisms.

Following birth, the lumen of the ductus arteriosus is gradually occluded by an overgrowth of its intimal tissue. The histological picture presented is somewhat suggestive of the changes seen in endarteritis obliterans. This process in the wall of the ductus is as characteristic and regular a feature of the development of the circulatory system as the formation of the cardiac septa. Its earliest phases begin to be recognizable in the fetus as the time of birth approaches, and after birth continue at an accelerated rate to terminate in complete occlusion of the lumen of the ductus about 6 to 8 weeks after birth. This progressive closure of the ductus arteriosus reduces the shunt from the pulmonary circuit to the aorta and, acting together with the newly assumed respiratory activity of the lungs themselves, gradually raises the pulmonary circulation to full functional level. Barcroft (personal communication) is inclined to believe on the basis of recent experiments that there is also contraction of the smooth muscle in the wall of the ductus following birth. If this proves to be the case it would mean that the increase in pulmonary circulation is accelerated by a physiological mechanism which begins to act more promptly than the mechanism of morphological closure. It is difficult to conceive of such muscular action closing the ductus immediately and completely and being effectively maintained during the 6 to 8 weeks occupied by morphological closure. Nevertheless such a vasoconstriction might well reduce flow through the ductus sufficiently to accelerate the increase in blood flow to the lungs and to facilitate the ultimate closure of the ductus arteriosus by the growth of its own intimal tissue.

The results of increased pulmonary circulation with the concomitant increase in the direct intake of the left atrium are manifested secondarily at the foramen ovale. Even before birth—in the latter part of fetal life as the lungs attained considerable development—we noted that a reduction in transseptal flow was beginning to be evidenced. Following birth, as the pulmonary return increases still more, compensatory blood flow from the right atrium to the left decreases correspondingly. This is indicated anatomically by a progressive reduction in the looseness of the *valvula foraminis ovalis* and the consequent diminution of the interatrial communication to a progressively narrower slit between the *valvula* and the septum. This first phase in the closure of the foramen ovale occupies approximately the 1st postnatal month, during which time the pulmonary return is mounting toward equivalence with the right atrial intake. When this equalization has occurred, the compensating one-way valve at the foramen ovale falls into disuse. Although a probe can still be passed freely behind the *valvula*, the foramen ovale may be regarded as functionally closed when this new intracardiac balance has been attained.

Then follows a period of 6 to 8 months in which the connective tissue of the *valvula* increases from 600 to 700 per cent (Patten, 1931). Probe-patency still persists but the size of the slit through which a probe may be passed progressively diminishes and the resistance to its passage increases with the increase in the thickness of the *valvula*. This second phase in the closure of the foramen ovale with its characteristic histological alteration is essentially the conversion of an originally movable, flap-like valve into a fixed septal structure.

Finally, coming leisurely in the wake of functional abandonment and as a culmination of the period of connective tissue proliferation, is the adhesion of the *valvula* to become an integral part of the interatrial septum. There is great individual variability in the age at which this final step in the closure of the foramen ovale occurs. A usual range, rather than a specific time of final anatomical closure, is all that can be specified. Substantiated cases of the fibrous adhesion of the *valvula* to the septum becoming complete under 3 months are exceedingly rare. The usual time of complete anatomical closure appears to be not earlier than the last 3rd of the

1st year after birth, and is frequently much later (Patten, 1931).

In 20 to 25 per cent of adult individuals the fibrous adhesion is never entirely completed (Table I). Provided the valvula amply

TABLE I

Records as to Completeness of Closure of Foramen Ovale, from a Large Series of Individuals Beyond Childhood

Observer	No. of cases examined	Not completely closed
Adami-Abbott, 1915	1374 (adults)	199
Bizot, 1837	155 (mostly adults)	44
<i>Brit. Anat. Soc.</i> , 1897 (Parsons and Keith)	316 (all above 10 yrs.)	76
Fawcett and Blachford, 1901	306 (all over 6 yrs.)	96
Hinze, 1893	359 (all over 20 yrs.)	82
Ogle, 1857	62 (adults)	13
Rostan, 1884, and Zahn, 1889	711 (661 over 20 years)	139
Seib, 1934	500 (all over 20 yrs.)	85
Wallmann, 1859	300 (291 over 20 yrs.)	130
Totals	4083	864

Foramen ovale not completely closed in 21.2 per cent of cases.

The exact percentage incidence of unclosed foramen ovale obtained by compiling such data naturally varies with the length of the series of cases and the criteria used in selecting acceptable data. In a previously compiled table for about 4000 cases, "mostly adult" but not rigidly selected for age, the per cent obtained was 24.6 (Patten, 1931). In a compilation of 2648 cases in which all cases under 20 years were excluded, Seib (1934) arrived at a figure of 23.1 per cent. The present table showing 21.2 per cent differs from my own previous one in the substitution of Seib's new series of 500 cases in which the ages were all known to be above 20 years, for the 500 cases of Klob in which no account was taken of ages, and which one suspects from the 45 per cent of non-closures must have included many very young individuals. The present table differs from Seib's in containing a considerably greater number of cases because of less rigid age selection. The point to be emphasized is the essential consistency of these three tabulations, rather than their minor variations. For all practical purposes we may say that in individuals beyond childhood we may expect 1 case out of every 4 or 5 to show an incompletely closed (*i.e.* "probe-patent") foramen ovale.

overlaps the foramen ovale such failures of complete adhesion appear to be no functional handicap to an otherwise normal individual. Because of this fact and the frequency with which they occur, these cases may well be regarded as variations of the normal rather than as abnormalities. Such an attitude, however, must be tempered by the realization that in the event of disturbances in the pulmonary circuit sufficiently severe to unbalance intra-atrial pressures, an area of incomplete adhesion may again become a path for transseptal flow. The interesting experimental work of Gross (1934), in which he observed the behavior of interatrial septa obtained at autopsy and clamped between artificial atria in which the pressures could be varied at will, clearly demonstrates that this is more than a mere theoretical possibility.

With this brief sketch of prenatal and neonatal conditions as a background we may turn to a consideration of the various types of developmental defects which may manifest themselves at the foramen ovale.

CONGENITAL DEFECTS AT THE FORAMEN OVALE

Congenital defects of the heart are commonly attributed to one of two alleged causes: to "developmental arrests" which are said to leave some essential cardiac structure in an "underdeveloped" condition characteristic of a phase of its formation during embryonic life; or to the damaging effects on local growth of some inflammatory process that becomes established during fetal life. Neither of these factors adequately accounts for the wide variety of congenital defects encountered either at the foramen ovale or other locations in the heart.

As far as the evidence from any material that I have seen is concerned, pathological lesions rarely, if ever, appear to play a part in the primary causation of a developmental defect at the site of the lesion. Inflammatory reactions resembling those caused in the adult by endocarditis undoubtedly do occur occasionally at the site of congenital defects. There is, however, no reason to believe that such a process is the cause of the congenital defect. On the contrary, the lack of any semblance of constancy in the association of such lesions with developmental defects in general points very strongly to the conclusion that the association, when it does occur, is fortuitous. Possibly a defect of such a nature that it constitutes

a point of local stress, as for example pulmonary stenosis, may furnish a site of predilection for an inflammatory process, once the causative agent has become established in the fetal blood stream. That a localized inflammatory process causes a developmental defect at the site of the lesion appears to be unsupported by any valid evidence.

While local pathological lesions may be discounted, or even dismissed altogether, as direct causative agents, congenital defects which might be interpreted as developmental arrests unquestionably occur. If, for example, septum secundum does not grow to the usual extent, the orifice to be occluded by the valvula foraminis ovalis remains abnormally large and, therefore, may be inadequately guarded by a valvula which is itself perfectly normal (Fig. 5). In such a case we might properly employ the expression "developmental arrest," for growth progressing along its normal course has fallen short of completion.

There are, however, defects at the foramen ovale that are in no sense the result of the cessation of a growth process short of its usual culmination. If, for example, the normal process of resorption which is concerned in the establishment of the secondary opening in septum primum (Text-Fig. 4, C) does not cease at the proper point, septum primum may be so extensively destroyed that it fails to occlude effectively a foramen ovale of normal size (Figs. 3 and 4). This is a radically different process from a developmental arrest. Instead of dealing with a growth process which has not gone to completion, we are dealing with a process of resorption that has gone too far.

Another condition which is a variant of the process just considered occurs not infrequently. The resorption of septum primum may take place in abnormal areas as well as to an abnormal degree. Instead of being limited to the quadrant in which the secondary opening in septum primum is ordinarily established, the resorption may occur in several places and progress to such an extent that the remains of septum primum can not possibly act as an efficient valve at the foramen ovale (Fig. 6). This abnormal resorption may start from the margins, as in the heart shown in Figure 6, or it may appear also in the form of multiple small openings reminiscent of the manner in which ostium II is first formed in septum I (Text-Fig. 4, C). The openings may be formed near the normal site of

ostium II or at various other parts of the valvula as shown in Figures 7, 8, 10, 12. In such cases we are dealing with the distortion of a resorptive process instead of with the "arrest" of a growth process. When it progresses and terminates normally, this process of resorption plays just as important a part in moulding an efficient valve as do the growth processes with which it is correlated.

In rare instances hearts are encountered that show no trace of a valve covering the foramen ovale (Fig. 16). While it is impossible to be certain that this represents a defect due to secondary resorption of a once present septum primum, circumstantial evidence points to that conclusion. When septum primum is primarily defective there remains a very characteristically shaped opening just above the atrioventricular valves. Usually the valves themselves are notched at the point where septum primum would have fused with the atrioventricular canal cushions. The absence of such a condition, in this heart, and the existence in other hearts of a whole series of conditions grading toward complete destruction of the free part of the valve (Figs. 1-14) point strongly toward secondary absorption of a once present septum primum as the correct interpretation.

The most common type of defect at the foramen ovale appears to be that in which there has been just a little too much resorption of septum primum at the normal site of ostium II (Fig. 3). This condition is surprisingly frequent in newborn infants. In a series of 100 consecutive cases studied with special reference to this condition its incidence was above 20 per cent. Apparently such slight failures of the valvula to overlap the foramen are rapidly compensated for in some manner because, except in newborn infants, they are not strikingly common. It may be that septum secundum grows somewhat after birth thereby reducing the extent of the foramen ovale and eliminating the small unguarded area. It is possible, also, that the marked fibrous development characteristic of the valvula from the 2nd to the 9th month after birth may account for the elimination of this defect in some cases. In 1 unusual case, secondary growth of the tissue around the limbus fossae ovalis was very marked and there had been, also, as far as one could judge by looking at the completed process, some secondary filling in of small multiple defects in the valvula (Figs. 20, 21, 22). How common such repair may be it is impossible to guess. The case

mentioned is the only one of the kind I have seen but it seemed unmistakable in its significance.

Probably the rarest of anomalies occurring at the foramen ovale is congenital atresia (Corvisart, 1818; Smith, 1846; Osler, 1880; and Lehman, 1927). Through the courtesy of Dr. Howard T. Karsner I had the opportunity of seeing the additional case illustrated in Figures 17, 18 and 19. Such cases throw an interesting side light on the functional significance of the foramen ovale during fetal life, for in every instance the left ventricle was developed to only about half its normal size. The muscular development of the ventricles being largely influenced by the volume of blood that they handle during their period of growth, one must infer that the half-normal development the left ventricle acquires in cases where the foramen ovale is prematurely closed depends on the blood returning to the left heart through the fetal lungs. The condition seen in these cases seems to corroborate the interpretation given above on the basis of orifice measurements, that approximately half the blood entering the left side of the heart in a term fetus comes by way of the lungs and half by way of the foramen ovale.

From the morphogenetic standpoint, congenital stenosis or atresia of the foramen ovale presents yet another different type of departure from the normal. It is not the result of inhibited growth, nor yet of exaggerated or distorted resorption. On the contrary, it is the continuation of a normal constructive process "beyond the point specified in the plans." Septum secundum fails to cease growing when its margins have reached the usual boundaries of the foramen ovale. Its growth continues abnormally until it has closed an opening without which the left side of the heart develops so defectively that it cannot long maintain the load imposed on it after birth.

That congenital defects at a given location may arise in such fundamentally different ways would seem to have significant implications. Cases have been here presented which show abnormal interatrial openings appearing as the result of: (1) underdevelopment of septum secundum; (2) resorption of septum primum starting in the normal location but going too far; (3) resorption of septum primum taking place in abnormal locations; and (4) overgrowth of septum secundum. Such radical differences in the

immediate mechanisms concerned should give us pause in considering any "blanket explanation" of congenital defects. Certainly the ultimate solution of the intricate problem of their causation will not be advanced by overemphasizing the developmental arrest concept when congenital defects may equally possibly be the result of a resorptive process which has gone astray, or a growth process which has failed to stop soon enough. Pending the acquisition of more satisfactory knowledge as to etiology, we would be on sounder ground if we were more restrained in our use of "developmental arrest" with its often false implications as to causation, and employed some such non-committal expression as developmental distortion or developmental defect.

CLINICAL SIGNIFICANCE OF DEFECTS AT THE FORAMEN OVALE

It would carry me out of my province to undertake any extensive discussion of the clinical problems presented by individuals with congenital defects at the foramen ovale. There are, nevertheless, certain things that stand out from a study of the records of a large number of cases that it might not be out of place to mention.

The still rather widespread practice of attributing otherwise unaccounted for deaths of young infants to "an open foramen ovale" is utterly unsound. In the first place, anatomical closure of the foramen ovale does not ordinarily take place until toward the close of the 1st postnatal year. Secondly, from 20 to 25 per cent of all adults show incomplete fusion of the valvula to the septum without the slightest evidence that this condition is any functional handicap, provided the valvula adequately covers the foramen and there are no other concomitant circulatory disturbances. Finally, the way individuals with extensively unguarded interatrial openings frequently live into maturity and even old age clearly shows the absurdity of regarding a mere unclosed foramen ovale as the immediate cause of a fatal circulatory disturbance.

While an unclosed but competent valvula foraminis ovalis cannot be regarded as a causative factor in circulatory disturbances, it frequently is the result of disturbances elsewhere. If, during the period in which the valvula would normally fuse to the septum, there is any condition operative that reduces the left atrial intake from the lungs, transseptal blood flow from right to left will continue to take place postnatally as it did prenatally and the

valvula will thereby be prevented from fusing to the septum. The most striking cause of such a situation is congenital pulmonary stenosis. Almost without exception when there is a pulmonary stenosis of embryological origin, an unfused and slack valvula persists at the foramen ovale. This is the logical sequel of the failure to accomplish the balancing of direct atrial intakes which normally comes with the attainment of full functional activity by the pulmonary circuit.

While it may not invariably be the case, there is usually a recognizable morphological difference between the condition exhibited by a valvula which has been kept open by circulatory unbalance and one which, although subjected to no such disturbance, has failed to fuse with the septum. In the fortuitous failure of complete adhesion which is encountered in 20 to 25 per cent of all adults the valvula tends to lie tight against the septum and the areas of incomplete fusion may be entirely overlooked unless one meticulously explores all parts of the valve margins with a fine flexible probe. When transseptal blood flow has persisted, the valvula tends to retain a certain fullness like that characteristically present in the fetus. It is likely for the same reasons to lie less closely against the septum, thus readily revealing the presence of an interatrial communication. These differences appear most clearly when a fresh heart is examined under water, with the aid of a current from a syringe.

There is a rather neglected corollary to the proposition that a foramen ovale with a freely opening valve should be regarded as part of the picture one would expect to find with congenital pulmonary stenosis. Cases of pulmonary stenosis not infrequently come to autopsy in which conditions at the pulmonary outlet alone do not give satisfactory evidence as to whether the stenosis was congenital or acquired. In such cases conditions at the foramen ovale may furnish valuable collateral evidence. In the absence of some other circulatory by-pass of similar functional significance, a completely closed foramen ovale in a case of pulmonary stenosis is strong evidence that the stenosis developed after the fetal-neonatal period.

Thus it should be emphasized that, when the foramen ovale possesses a competent valve, the question of whether or not this potential by-pass is closed should be considered in the light of the

factors involved in establishing and maintaining a balanced atrial intake. Phrased in another way, an open foramen ovale of this type is not to be thought of as a cause of circulatory disturbances but as a result of them. Equally obviously, the question of the condition of the pulmonary circuit should be the first consideration from the standpoint of diagnosis. The fundamental importance of maintaining a right to left transatrial blood flow during fetal life is correlated with the low volume of the pulmonary circuit while the lungs are not functioning in respiration. The cessation of transatrial blood flow and the balancing of direct atrial intakes, which occurs following birth, is dependent on the attainment of full functional level in the pulmonary circuit. And finally there is no single factor as likely as some disturbance of the pulmonary circulation to upset this balance during postnatal life and thus reopen a foramen ovale which has happened to remain unsealed.

A heart with a foramen ovale which is inadequately guarded by a malformed valvula (Figs. 14 and 15), or one which is completely unguarded (Fig. 16) presents quite different possibilities from cases of the type just discussed. In hearts with a competent but unfused valvula, transseptal flow takes place readily from right to left but is inhibited from left to right. When there is an unguarded foramen ovale, transseptal flow can take place just as readily from left to right as from right to left. Of course if there is an associated pulmonary stenosis causing a compensatory flow from right to left, the mechanism of the circulation will be quite similar to that just discussed for a heart with a competent but unfused valvula. If, on the other hand, the pulmonary circuit is normal a radically different picture is presented. The clinical manifestations of such cases have recently been presented very ably and in considerable detail by Roesler (1934). When, as Roesler has done, all cases of interatrial defect in which there is an associated pulmonary stenosis are ruled out, a very characteristic picture remains. Its outstanding features, summarized from Roesler's findings, are essentially as follows: The heart tends to be enlarged, often becoming of enormous size and causing a marked precordial bulge. The enlargement involves primarily the right side of the heart and especially the right ventricle which is usually both extensively dilated and hypertrophied. The left ventricle remains strikingly uninvolved by the enlargement (Abbott's Case

1, 1915, with hypertrophy involving the left as well as the right ventricle is exceptional). The pulmonary artery is consistently larger than the aorta, the average ratio being 3:2. The aorta tends to be below normal size and thin walled. In upwards of three-fourths of the long-standing cases valvular lesions were found which affected predominantly the mitral orifice (see also McGinn and White, 1933). In contrast with certain other types of congenital defects, subacute bacterial endocarditis is strikingly absent, and chronic pericardial disease, crossed embolism, and pulmonary tuberculosis are noticeably rare concomitants.

The critical factor in this picture again appears to involve relative atrial intakes and pressures. In normal adults the pressure in the left atrium is believed to be slightly greater than that in the right. Under such conditions an unguarded interatrial opening would permit the backing of blood from the left atrium into the right, thus causing the right side of the heart to handle an increased amount of blood. This situation acting over a long period of time would account for the dilatation and hypertrophy of the right ventricle and the large pulmonary arteries which are so characteristic in these hearts. The same transatrial flow which overloads the right heart, by reducing the blood entering the left ventricle, would account for the fact that it is consistently uninvolved by dilatation or hypertrophy, and also for the fact that the aorta tends to be relatively small.

Detailed physiological evidence for this interpretation is admittedly scanty but the circumstantial evidence is convincingly consistent. For a fuller discussion from a clinical standpoint reference should be made to Roesler's excellent paper. There are, however, two points of special interest which it might be pertinent to mention here. One is the striking absence of crossed emboli in cases of frankly unguarded interatrial openings where the flow is presumably taking place from left to right, in contrast with the comparative frequency of crossed emboli in association with pulmonary stenosis where the interatrial communication may be much smaller but where the leakage is from right to left. The other point concerns cyanosis. Characteristically, as long as the individual is subject to no complicating factors, cyanosis will be absent as one would expect on the basis of the above interpretation of a left to right shunt. A sudden terminal cyanosis ("cyanose tardive,"

Bard and Curtillet, 1889) is, however, very likely to occur. What apparently happens in such cases is a reversal of the direction of the shunt due either to intercurrent pulmonary difficulty, or to breakdown of the long overloaded right ventricle.

Many individuals, even with large interatrial defects, live to advanced years with surprisingly little handicap. Perhaps the most interesting are cases in which individuals have performed hard work for many years or otherwise lived an active life. Caton (1878) wrote of a man, powerfully built, who had been a seaman for 20 years and finally died of an acute respiratory infection at the age of 40. His interatrial defect was 3 inches in diameter. The heart here illustrated in Figure 14 was from a charwoman who lived to the age of 52, and that illustrated in Figure 16 was from a day laborer who lived to the age of 44. Gibier (1880) records the case of a man who showed no cardiac symptoms during life, withstood anesthesia for a cancer operation and lived to the age of 70. Firket (1880) reported on a woman who had 11 children, and lived to an age of 74 years. Tarnower and Woodruff (1936) have published the clinical findings and detailed autopsy report on a woman who lived to be 77 years of age although she had a patent foramen ovale measuring 4 cm. in diameter. Why this tolerance of the defect is so striking in some cases and why other individuals with similar defects are incapacitated and go on to a relatively early death from cardiac failure is difficult to explain. One significant fact brought out by Roesler, is that in three-fourths of the 62 cases of unguarded interatrial defects which he reviewed chronic valvular lesions of some degree were found. This is considerably above the average incidence of such lesions in all types of congenital defects, which is placed by Abbott (1932) at 17.6 per cent. The mitral orifice seems to be the one most frequently affected in individuals with interatrial defects. While there is no clue as to why this is the case the aggravating results of its occurrence are self-evident. Either mitral insufficiency or stenosis will tend to exaggerate the flow from left atrium to right which is the critical factor in producing the characteristic cardiac changes seen with an interatrial defect, whether it be at an unguarded foramen ovale or at the site of interatrial foramen primum. Apparently when this back flow becomes extreme there is a breakdown of the compensation previously maintained and the individual begins to show

marked cardiac symptoms which are likely to increase rather rapidly in severity.

As far as it is possible to generalize, the situation would appear to be that even a large interatrial defect is by itself not necessarily incompatible with a long and active life. It does, however, produce striking and characteristic changes in the proportions of the heart. Moreover, such hearts appear to be more than normally vulnerable. Individuals who have carried the defect for years without particular handicap may suddenly show signs of cardiac failure. What part is played in such cases by the frequently concomitant valvular disease, why valvular disease has such a high incidence in these hearts, and why it shows a predilection for the mitral orifice are all matters that need further study.

SUMMARY

This paper aims to present a survey of the various types of developmental defects encountered at the foramen ovale, and to make a re-evaluation of their significance. As a foundation for this study the formation of the interatrial septal complex in the embryonic heart is reviewed. In this review special attention is given to the functional significance of the three different interatrial communications which appear during intrauterine life.

The mechanism of the closure of the foramen ovale following birth, and the time at which its closure may be expected to occur, are presented in the light of recent work which points to the conclusion that the postnatal changes in circulation are far less abrupt and immediate than traditionally postulated.

The various types of developmental defects which may occur at the foramen ovale are illustrated and the embryological processes which have been distorted in the production of each type of defect are discussed. It is pointed out that the "developmental arrest" concept is inadequate as an interpretation, in view of the several fundamentally different ways in which the developmental defects may arise.

Finally, brief comment is made on certain things of clinical interest that emerge from the review of a large number of cases. Reasons are given for regarding as unsound the still widespread practice of attributing otherwise unaccounted for deaths of neonatal infants to "an open foramen ovale."

Emphasis is placed on the necessity of discriminating more critically between an open foramen ovale with a competent but unfused valve, and a frankly unguarded foramen ovale with an incompetent valve. With an unfused but competent valve transseptal leakage, if it occurs at all, is limited to one direction — right to left. The common cause of such leakage is some disturbance of the pulmonary circuit which results in relative lowering of left atrial intake and pressure. What occurs at the foramen ovale in such cases should be regarded as a result of disturbances elsewhere and not as a cause.

In sharp contrast with such cases are those in which the foramen ovale is inadequately guarded by an incompetent valve. In these cases, provided the pulmonary circuit is normal, transseptal flow appears to take place quite consistently from left to right. This overloads the right heart at the expense of the left and causes characteristic changes from the normal cardiac proportions. There tends to be a marked dilatation and hypertrophy resulting in a great increase in heart weight. The right side of the heart, especially the right ventricle, is most conspicuously involved, whereas the left ventricle remains strikingly uninvolved. Consonant with the relative ventricular development, the pulmonary artery is markedly larger than the aorta, which tends to be below normal size and thin walled. In these cases with an unguarded foramen ovale the characteristic and clinically recognizable changes in cardiac structure are clearly the result of the defect. Even in these cases the prognosis should not be unduly pessimistic as many individuals support such defects surprisingly well and live to an advanced age.

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DESCRIPTION OF PLATES

PLATE 30

- FIG. 1. Usual appearance of the *valvula foraminis ovalis* as seen from the left in the heart of a newborn infant. Note the fullness of the *valvula* which is represented in the bulged out position it assumes when subjected to excess fluid pressure from the right atrium through the *foramen ovale*. The size and position of the *foramen ovale* are indicated by the broken line.
- FIG. 2. Resorption of *septum primum* in an abnormal area dorsal to the usual site of *ostium secundum*. In this instance the abnormality is of no functional significance since it does not unguard the *foramen ovale*.
- FIG. 3. Slight incompetence of *valvula foraminis ovalis* due to excess resorption of *septum primum* at the normal site of *ostium secundum*. This is a common condition, occurring in some 20 per cent of newborn infants. It is apparently "corrected" in most cases by postnatal changes either in *septum primum* or *septum secundum* and probably has no functional significance.
- FIG. 4. Incompetence of *valvula* due to excess resorption at the normal site of *ostium secundum* combined with resorption at an abnormal site. This defect is definitely more extensive than the apparently correctable type shown in Fig. 3, and undoubtedly would persist throughout life.
- FIG. 5. The *valvula* is abnormally resorbed in two small areas but these defects are so slight that they would be of no significance were they not combined with an abnormally large *foramen ovale*. The large *foramen ovale* due to defective development of *septum secundum* is the condition of primary importance in this case.
- FIG. 6. Extensive defects of the *valvula* due to over-resorption in the normal and in several abnormal locations.

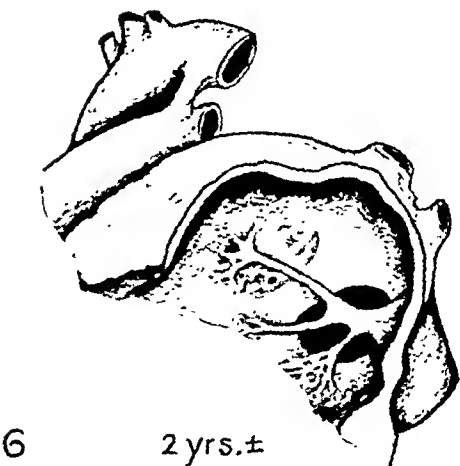
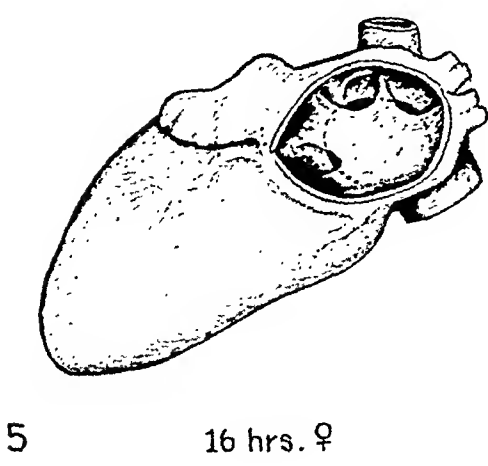
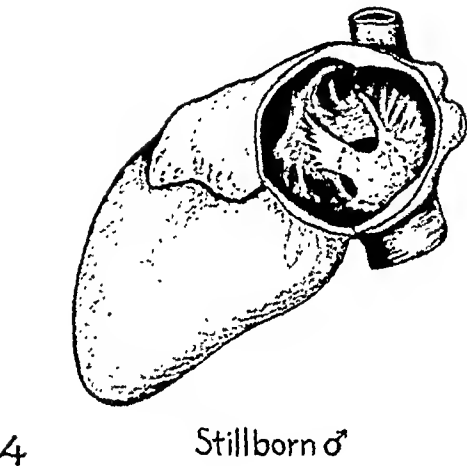
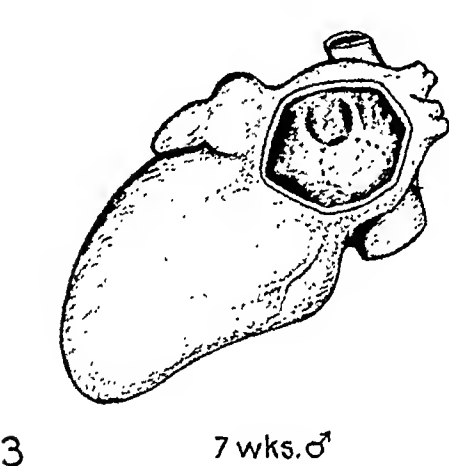
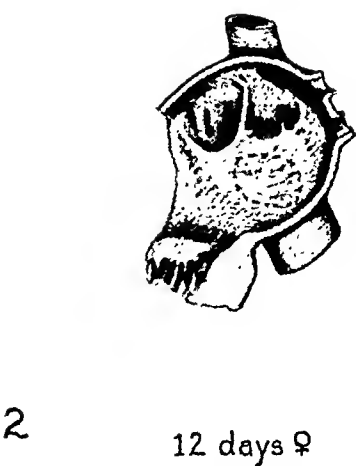
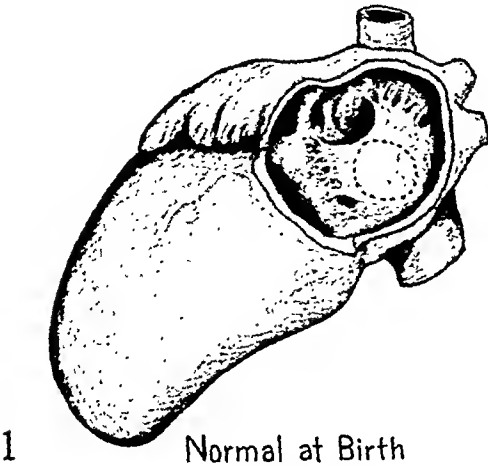


PLATE 31

- FIG. 7. Multiple small perforations of valvula. The formation of ostium secundum normally starts with the appearance of small openings which later coalesce. Here such openings have appeared in a definitely abnormal location.
- FIG. 8. Case similar to that shown in Fig. 7, except that the openings are larger and more widely distributed.
- FIGS. 9, 10 and 11. Various combinations of marginal over resorption of the types shown in Figs. 1-6 with small multiple perforations similar to those shown in Figs. 7 and 8.
- FIG. 12. Extreme resorption of valvula combined with abnormally large foramen ovale due to defective development of septum secundum. There is also in this heart unbalanced development of the ventricles, the left ventricle being very small, correlated probably with a defective pulmonary circuit as indicated by the marked stenosis of the pulmonary veins.

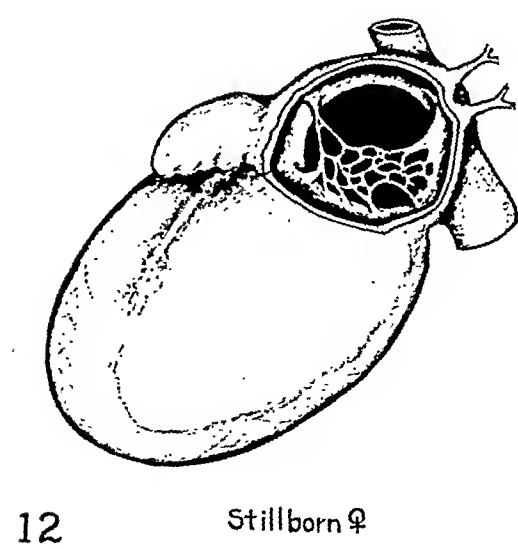
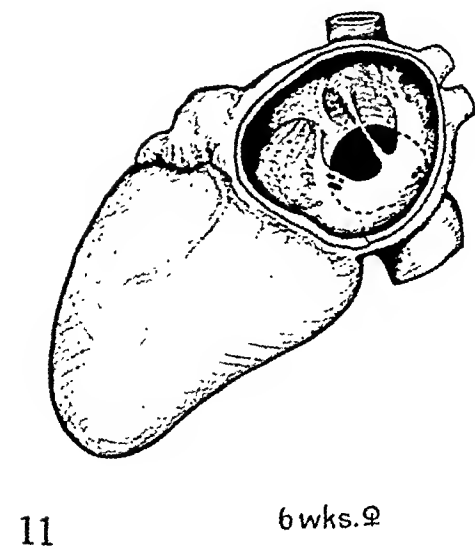
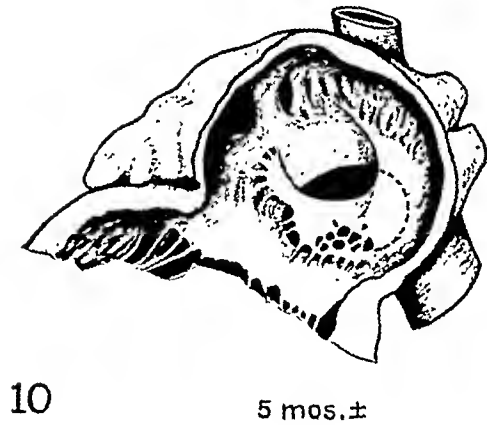
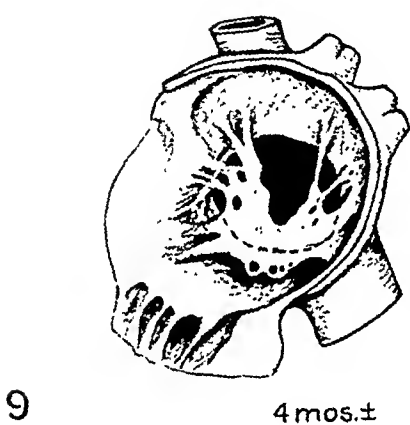
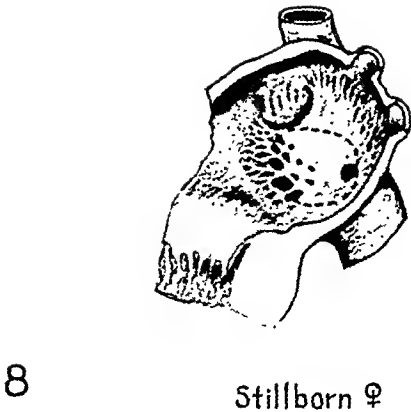
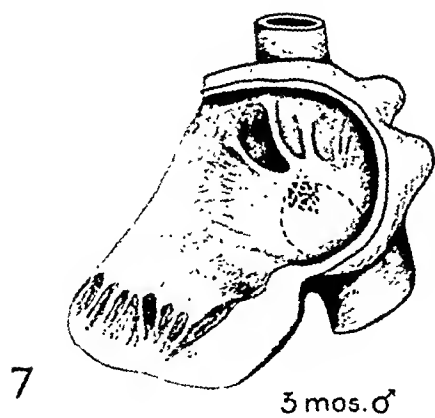
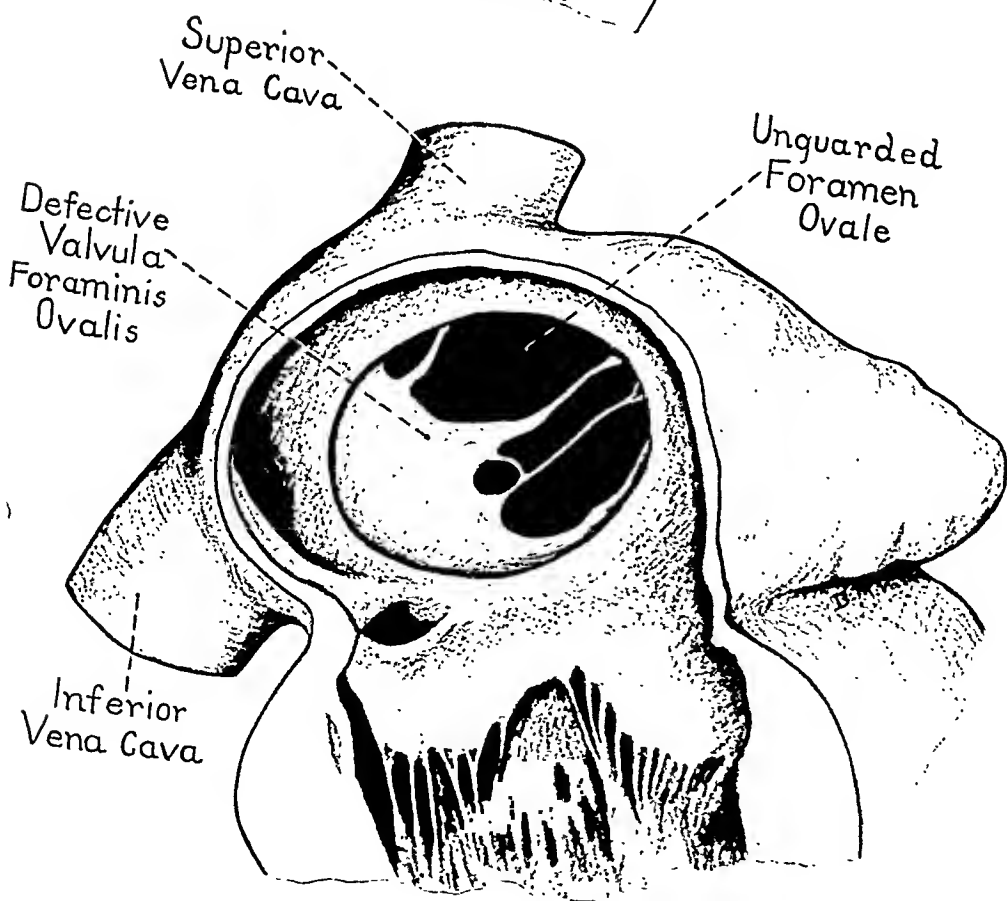
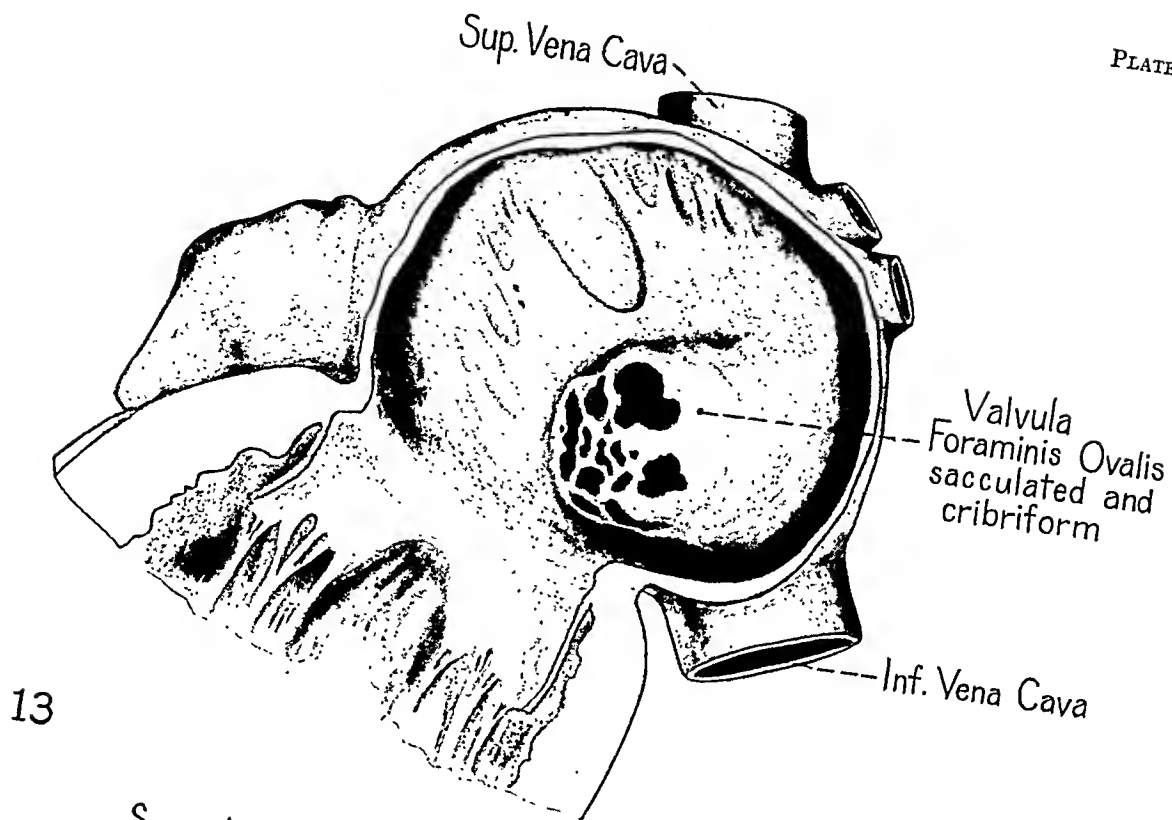


PLATE 32

FIG. 13. Valvula foraminis ovalis markedly sacculated toward left atrium and showing multiple perforations of considerable size. No clinical history. Dissecting room specimen "from an old man" sent in by Dr. John Donaldson, University of Pittsburgh.

FIG. 14. Drawn from specimen No. 3027, Pathologisch-Anatomisches Institut, Vienna. The heart was from a charwoman who died suddenly of pulmonary thrombosis at the age of 52 years. Rokitansky (1875, p. 52) gives a brief unillustrated record of the case. The heart was "very large, 90 mm. long and 115 mm. broad" with rounded apex. The similarity of the morphological picture presented by this adult heart and the infant heart shown in Fig. 11 is interesting.



Developmental Defects at Foramen Ovale

PLATE 33

FIG. 15. Drawn from specimen No. 2410, Pathologisch-Anatomisches Institut, Vienna. Case briefly described by Rokitansky (1875, p. 47). Day laborer 21 years old, admitted to the hospital with "the itch" (Krätze). Died following an unexpected attack of dyspnoea. Heart very large, "100 mm. lang und ebenso breit"; right ventricle and conus "erweitert." Heart weight not recorded.

FIG. 16. Drawn from specimen No. 2225, Pathologisch-Anatomisches Institut, Vienna. Case mentioned briefly by Rokitansky (1875, p. 45). Male, day laborer, 44 years old. Had purulent bronchitis and gangrene of oral mucous membrane. No cyanosis noted. Immediate cause of death appeared to be primarily pulmonary, although the clinical information given is too meager to be certain. The heart was greatly enlarged and showed a fibrinous pericarditis. No trace of a valvula foraminis ovalis could be seen and the unguarded foramen ovale was of enormous size measuring 40 by 47 mm. in the fixed specimen.

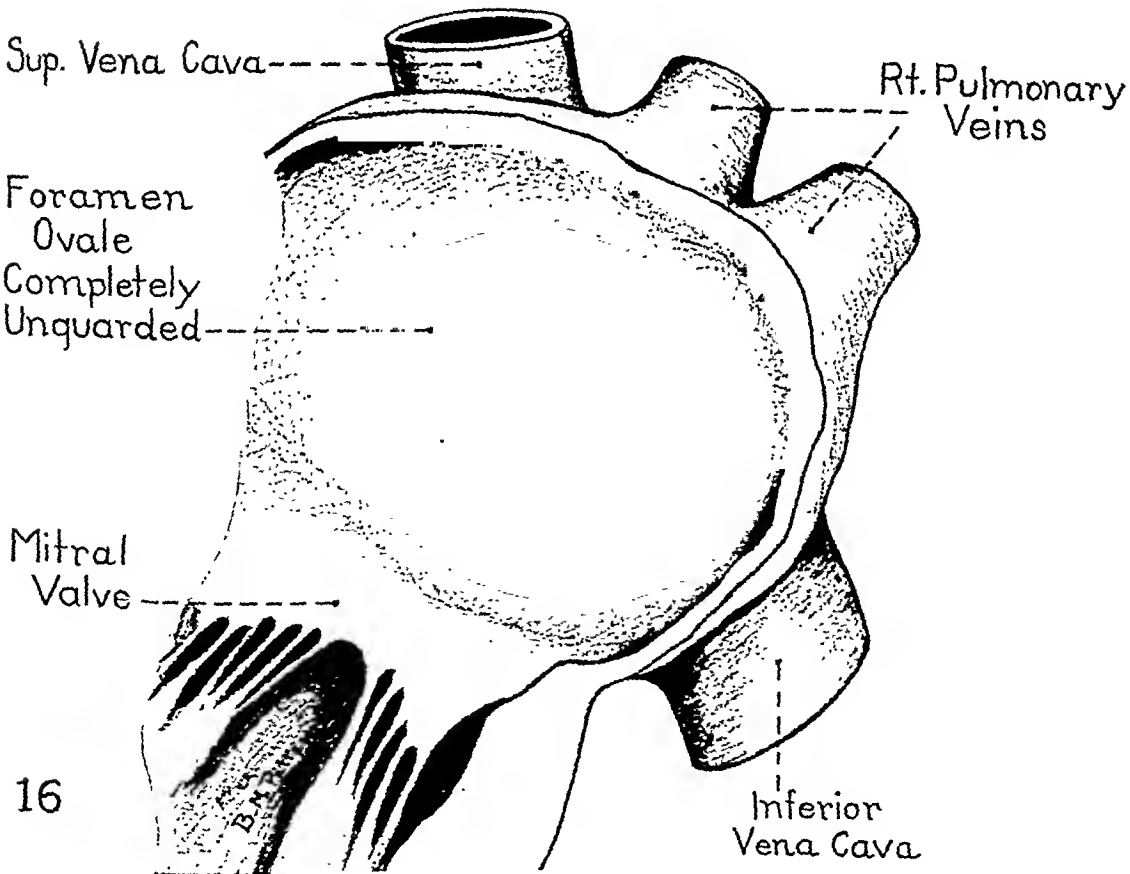
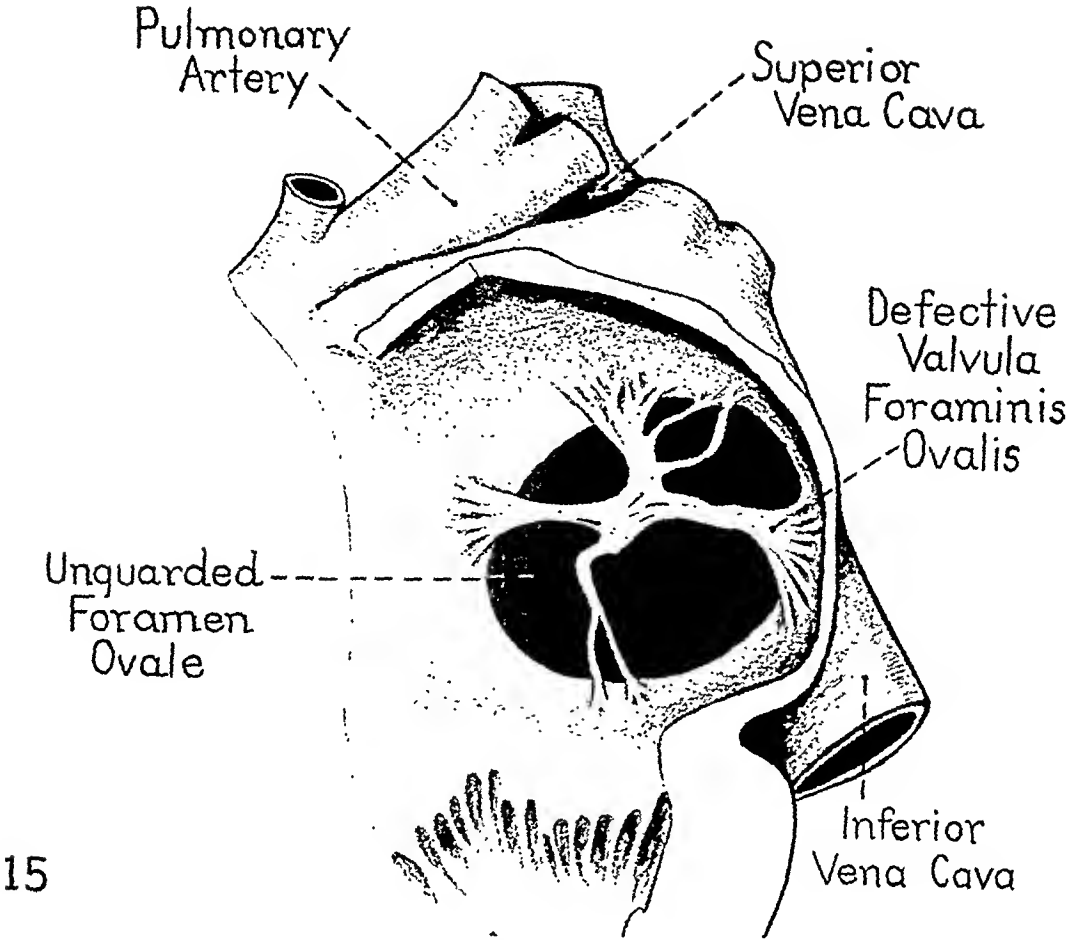
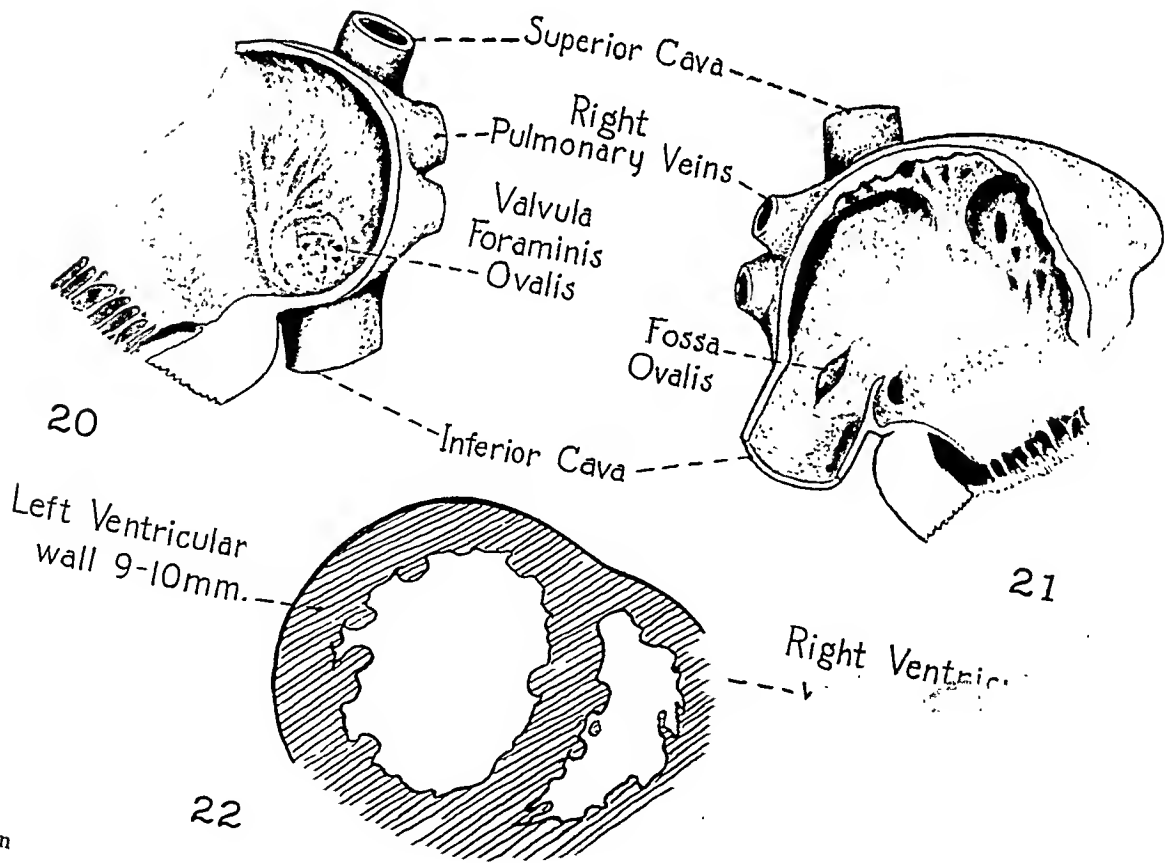
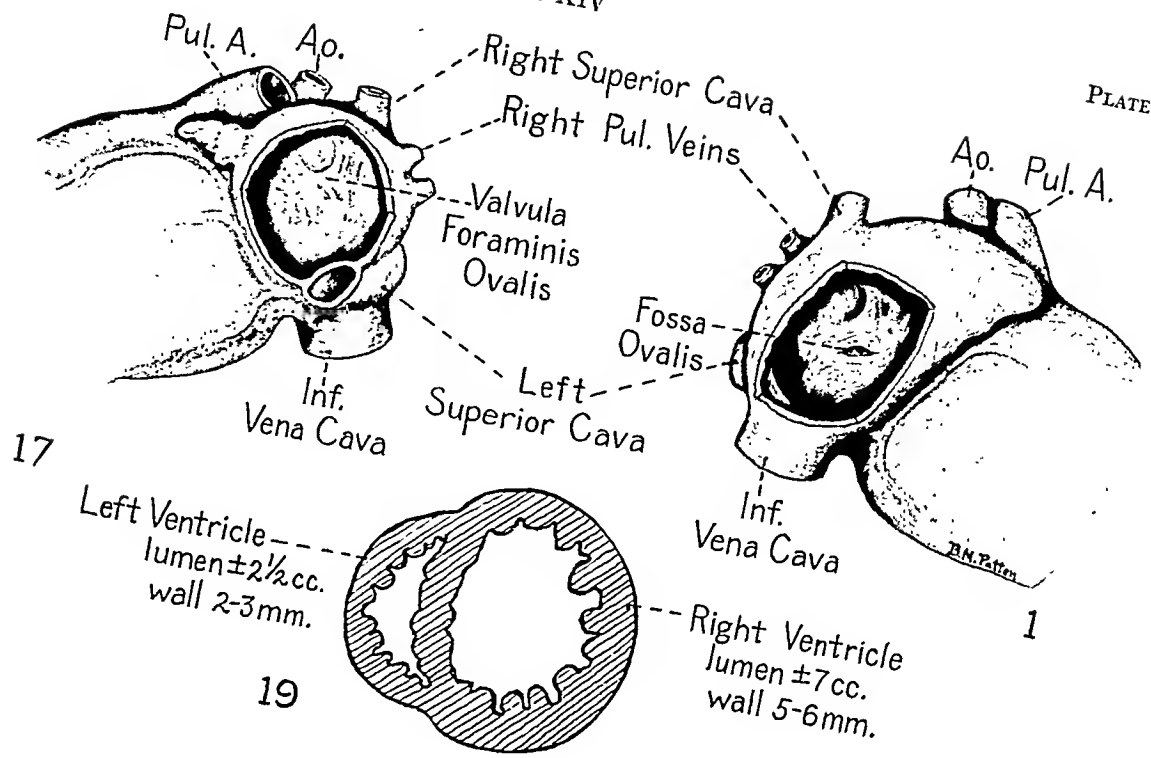


PLATE 34

FIGS. 17, 18 and 19. Heart of a 1 month old infant showing the conditions encountered in premature closure of the foramen ovale. (Babies and Childrens Hospital, Autopsy No. A-374, made available through the courtesy of Dr. Howard T. Karsner, Western Reserve University Medical School.) Although there is no way of being certain whether the closure as seen at autopsy had been fully established *in utero*, there seems no doubt that a marked ante natal stenosis, if not an atresia, must have existed. This is indicated: (1) by the slit-like fossa ovalis which is but a small fraction of the oval opening left when septum secundum normally ceases further growth; (2) by the complete adhesion of the valvula which does not ordinarily occur until several months after the cessation of trans-septal flow; and (3) by the deficiently developed left ventricle which seems clearly attributable to lessened left atrial intake due to a foramen ovale closed, or greatly narrowed, during the growth of the fetal heart.

FIGS. 20, 21 and 22. Possible case of postnatal repair of congenital defect at the foramen ovale. The appearance of the narrowed fossa ovalis is superficially somewhat similar to the case of premature closure illustrated above, but there are two associated conditions which indicate that in this case the narrowing occurred postnatally. First is the normal development of the ventricles. If the fossa ovalis had been of its present abnormally small size during intrauterine life the left ventricle would have been undersized. Second is the faint depression which sketches the contours of a fossa ovalis of the normal size. This seems to suggest that the fossa was, at the time of birth, of the size outlined by this depression, and that the differently disposed tissue now narrowing it was formed later. There is yet another interesting condition pointing in the same direction. In the valvula foraminis ovalis one sees an arrangement of robust strands which suggest that it might once have appeared not unlike the defective valve in Fig. 9. Between these heavy strands there is a more delicate tissue which might conceivably have been secondarily formed. Of course this entire interpretation must be regarded as tentative, but postnatal repair of a congenital defect, if it does occur, is of so much interest that it seemed worth while presenting this unique case in the hope of stimulating further observations bearing on such a possibility.





THE EFFECT OF SYPHILIS ON LOCAL TUBERCULOUS LESIONS IN RABBITS *

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The influence of syphilis on the course of other diseases, and other diseases on the course of syphilis, is not definitely known. Certainly the view held by John Hunter¹ that "no two actions can take place in the same constitution or in the same part at once and at the same time" is not tenable in the light of our present knowledge. Pearce² observed that the simultaneous inoculation of vaccine virus and syphilitic material into two different sites resulted in a more severe form of syphilis in rabbits, whereas the injection of the syphilitic material into rabbits previously immunized with vaccine virus gave rise to an infection with a less severe course. Pearce and Brown³ found that with one exception a transplantable malignant tumor of rabbits failed to grow in rabbits infected with syphilis. Chesney and Kemp⁴ noted that inflammation induced by trauma or by coal tar favored the multiplication of the *Treponema pallidum* and the extension of the syphilitic process.

As to the part played by syphilis in tuberculosis, there is a wide difference of opinion. Sergeant⁵ believed that syphilis predisposes to tuberculosis and creates a site of predilection for the tuberculous process. That active syphilis influences unfavorably the course of tuberculosis has been maintained by Norris and Landis,⁶ Fishberg,⁷ Habliston and McLane,⁸ Chadwick,⁹ and others. On the other hand, Petresco¹⁰ was of the opinion that there is a definite antagonism between the two. Weiss¹¹ could not establish any significant clinical relation between syphilis and tuberculosis. He concluded that the influence of syphilis on tuberculosis varies with the degree of the constitutional resistance of the individual.

EXPERIMENTAL

In order to determine whether syphilis influences the course of tuberculous lesions, a study of the gross and microscopic character

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of tuberculous lesions of the skin of syphilitic and of non-syphilitic rabbits was undertaken. This method of approach was chosen because it permitted observation of lesions in the same animals at varying intervals over a considerable period of time, thus minimizing the element of individual variation in resistance.

Method

Into the left testicle of each of 12 albino New Zealand rabbits, weighing from 2000 to 2500 gm., an emulsion of the testicle of a rabbit that had previously been infected with the Nichols strain of *Tr. pallidum** was injected. On dark-field examination this emulsion showed 4-5 spirochetes per high power microscopic field. Three to 4 weeks following inoculation the characteristic enlargement and swelling of the testes were observed, followed by prompt subsidence of the swelling and the appearance of indurated nodules. In all of the inoculated rabbits metastatic lesions were found in the opposite testicle. Nine weeks following the infection with *Tr. pallidum*, an injection of 0.1 mg. of a virulent strain of bovine type tubercle bacillus in 0.1 cc. of physiological salt solution was made into the skin over the abdomen at each of six widely separated points. At the same time 12 non-syphilitic rabbits of approximately the same weight and breed were injected in precisely the same manner with a similar amount of the bacillary suspension. All of the rabbits were maintained on a diet of prepared pellets, hay and water, with fresh greens twice a week. All animals continued to gain in weight until the 12th week, when a marked loss of weight was noted.

The resulting tuberculous lesions were studied macroscopically, and were then biopsied under ether anesthesia for histological study according to the following scheme: In a series of 3 syphilitic and 3 non-syphilitic rabbits a lesion was removed from each rabbit 1, 3, 5, 12, 24 and 48 hours after the injection of the tubercle bacilli; in a second series of 3 syphilitic and 3 non-syphilitic rabbits lesions were removed after 3, 4, 5, 6, 7 and 14 days; in a third series, specimens were taken after 3, 4, 5, 6, 7 and 8 weeks; and in a fourth series, after 9, 10, 11, 12, 13 and 14 weeks. A section of uninoculated skin was removed from each rabbit as a control.

* This strain was kindly furnished us by Dr. M. Severac of the Dermatological Research Laboratory, Philadelphia.

The sections removed at biopsy were embedded in paraffin, sectioned and stained with various stains, including hematoxylin and eosin, Ziehl-Neelsen stain for acid-fast bacilli and Mallory's aniline blue collagen stain.

Macroscopic Characteristics of Lesions

The local tuberculous lesions were found to vary in size, not only among the different rabbits but also at the different sites of injection in the same rabbit.

Tubercle formation occurred earlier and was, on the average, throughout the course of the experiment, from 2 to 5 mm. larger in diameter and more elevated in the syphilitic rabbits. In an occasional syphilitic rabbit, however, the lesion was no greater than that noted among the controls. Ulceration, which occurred among the non-syphilitic rabbits within the 1st week, did not occur until the 2nd week among those with syphilis. These ulcers, which were 2 to 8 mm. greater in diameter among the syphilitic animals, extended more rapidly and showed less tendency to heal than similar ulcers in the non-syphilitic. In both groups the ulcers tended to be sharply defined and crater-like, with undermined edges.

The variation in the time of enlargement, in size and in the occurrence and extent of caseation in the regional axillary and inguinal lymph nodes, was so great among individual rabbits as not to permit drawing any conclusions.

Pathological Histology

Sections of the uninoculated skin removed from the syphilitic and from the non-syphilitic rabbits showed no conspicuous histological differences.

Sections of the skin removed from both syphilitic and non-syphilitic rabbits 1 hour after the injection of 0.1 mg. of tubercle bacilli showed a moderate degree of swelling in the derma, some separation of the collagen fibers, and dilatation of blood vessels. Slight leukocytic infiltration, most marked in the papillary layer of the derma, and somewhat more intense in the syphilitic than in the non-syphilitic rabbits, was present. Polymorphonuclear cell phagocytosis of the tubercle bacilli, which at this period appeared singly and in small clumps, was noted in both groups of animals.

Sections of skin removed from the non-syphilitic rabbits 3 hours, 5 hours and 12 hours after inoculation showed in the widened and edematous tissue spaces a diffuse but moderate degree of infiltration with polymorphonuclear cells, as well as small aggregations of these cells about clumps of tubercle bacilli. The blood vessels were dilated, and a slight degree of diapedesis was observed. Phagocytosis of tubercle bacilli by polymorphonuclear cells was evident and numerous extracellular clumps of these microorganisms were seen.

In the sections of skin removed from the syphilitic rabbits 3 hours, 5 hours and 12 hours following the injection of tubercle bacilli into the skin, the blood vessels and lymphatics were markedly dilated and the degree of edema was greater than among the non-syphilitic rabbits. Numerous leukocytes were observed along the endothelial lining of the vessel wall, while dense aggregations of polymorphonuclear cells and amphophiles occurred about the vessels (Fig. 1). The lymphatic vessels were dilated and contained small numbers of lymphocytes in contradistinction to the lymphatic vessels of the non-syphilitic rabbits, which were inconspicuous. Phagocytosis of tubercle bacilli by polymorphonuclear cells was not conspicuously different from that observed in the non-syphilitic animals, except that in sections removed from the syphilitic rabbits 12 hours after infection, was observed a marked increase of mononuclear cells, many of which were phagocytic.

In sections of skin removed from the non-syphilitic rabbits at daily intervals during the 1st week following infection an increasing number of polymorphonuclear cells was observed, and amphophiles and some mononuclear cells of the macrophage type were seen scattered diffusely throughout the derma and subcutaneous tissue, and occurring occasionally as small aggregations about blood vessels, as well as about clumps of tubercle bacilli. Three to 4 days after infection there was noted a small abscess which rapidly extended and which several days later ulcerated through the epidermis. The abscess consisted of dead and degenerated cells and collagen fibers (Fig. 2). Numerous tubercle bacilli, many extracellular and some intracellular, occurring in clumps, as well as dispersed, were noted within the abscess and to a lesser degree in other parts of the lesion. The abscess was surrounded by a moderate number of newly formed capillaries and a moderate dif-

fuse infiltration of mononuclear cells, amphophiles and fibroblasts.

In the lesions removed from the syphilitic rabbits during the 1st week following infection with tubercle bacilli, the histological changes were significantly different from those observed in the non-syphilitic rabbits. In these animals there were observed more or less widely separated, sharply defined aggregations of mononuclear cells massed about vessels and in some instances about newly formed capillaries (Fig. 3). These mononuclear cells had large, round, pale staining vesicular nuclei which, in some cells, showed indentations. An occasional polymorphonuclear cell and amphophile were observed in these aggregations. Between the collagen fibers were noted an increased number of fibroblasts as well as some infiltration with mononuclear cells. The blood vessels were dilated and numerous polymorphonuclear cells and amphophiles were observed along the endothelial lining.

The above described cell aggregations were first observed 48 hours after the injection of tubercle bacilli and became increasingly denser during the 1st week. In the syphilitic rabbits abscess formation was delayed, occurring 1 to 2 weeks after infection, and was less extensive than that noted among the non-syphilitic animals.

These lesions, because of the focal aggregations of large mononuclear cells about capillaries and the formation of fibroblasts and capillaries, resemble the lesions of primary and secondary syphilis. Figure 4 is a microphotograph of a primary lesion resulting from the injection 6 weeks previously of a suspension of *Tr. pallidum* into the skin of a normal rabbit.

In the lesions removed from the non-syphilitic rabbits 2 weeks after infection with tubercle bacilli there was an abscess that had ulcerated through the epidermis and was sharply defined by a zone of necrosis beneath which granulation tissue rich in capillaries and fibroblasts was present. A small number of epithelioid cells and some plasma cells were scattered throughout the granulation tissue. No acid-fast bacilli were found after prolonged search. In the lesions removed a week later the abscess and ulcer had extended and widespread edema separated the dead and degenerating polymorphonuclear cells from one another. Beneath the area of ulceration there was a diffuse infiltration with epithelioid cells, many of them with a large amount of foamy cytoplasm.

In the lesions removed from the syphilitic rabbits 2 weeks after infection, ulceration was more extensive than among the non-syphilitic rabbits and the slough was separated from the underlying tissue by a zone of deeply staining polymorphonuclear cells. Beneath this zone there was an extensive infiltration of mononuclear cells resembling those previously described in the syphilitic rabbits. These cells, many of which showed mitotic figures, occurred in aggregations among the collagen fibers. Similar aggregations occurred about the dilated vessels and about some of the capillaries. An occasional small aggregation of epithelioid cells was seen throughout the lesion, but acid-fast bacilli were not demonstrable. In the lesions removed a week later the slough was definitely more extensive and granulation tissue was more conspicuous. Epithelioid cells occurred in moderate numbers, not diffusely as in the non-syphilitic rabbits, but in aggregations. A number of small areas of caseation were observed scattered throughout the deeper layers of the derma.

Four weeks after infection increasing ulceration was present in the lesions removed from the non-syphilitic rabbits. Beneath the newly regenerating epithelium, which dipped downward into the derma and into the subcutaneous tissue, there was a diffuse infiltration of epithelioid cells of varying size (Fig. 5), many of which contained protoplasm of foamy appearance. Occasional small aggregations of lymphocytes and an occasional giant cell, as well as small collections of plasma cells, were scattered throughout the lesion. The walls of the blood vessels were thickened. No tubercle bacilli were demonstrable.

In the lesions removed from the syphilitic rabbits 4 weeks after infection the ulceration was more extensive than among the non-syphilitic rabbits and no regeneration of the surface epithelium was noted. A well defined layer of polymorphonuclear cells was present beneath the ulcerated area and adjoining this layer was an area of granulation tissue rich in fibroblasts and mononuclear cells (Fig. 6). In the deeper layers of the derma there was a marked increase of dense connective tissue, while the blood vessels were definitely thickened, but to no greater degree than among the non-syphilitic animals. Epithelioid cells as well as mononuclear cells were distributed, not diffusely, but rather as dense aggregations, frequently about blood vessels and separated from one another by

dense bands of collagen fibers. Several small areas of caseation were noted, as well as small aggregations of plasma cells and a small number of giant cells. No tubercle bacilli were demonstrable.

In the lesions removed from the non-syphilitic rabbits 5 and 6 weeks following infection with tubercle bacilli, the surface ulceration, while extensive, was less so than in the previous lesions. Regeneration and invagination of the surface epithelium were again noted. A single, sharply defined large tubercle consisting essentially of epithelioid cells, and occasional collections of small lymphocytes were seen beneath the ulcer. In the deeper layers of the derma as well as in the subcutaneous tissue there were areas of granulation tissue and a marked increase of collagen fibers. Several small caseous areas were seen, as well as occasional areas of softening infiltrated in some instances with polymorphonuclear cells, many of which were degenerated. A number of giant cells were found while plasma cells were numerous throughout. Tubercle bacilli were not demonstrable.

In the lesions removed from the syphilitic rabbits at this time the surface ulceration had progressed, while there was little or no regeneration of the surface epithelium. Extensive granulation tissue consisting of newly formed capillaries and fibroblasts was observed throughout the derma and to a lesser extent in the subcutaneous tissue. Aggregations of large mononuclear cells and plasma cells were present about some of the newly formed capillaries. Areas of caseation more extensive than among the non-syphilitic rabbits were noted, and in some of the animals there were small areas of softening infiltrated with degenerating polymorphonuclear cells. Epithelioid cells were few, and usually found at the margin of the granulation tissue. These cells, smaller than those observed in the non-syphilitic rabbits, frequently occurred in aggregations about blood vessels. Plasma cells and giant cells were numerous, the former occurring in small dense aggregations. No tubercle bacilli were found.

The lesions removed at weekly intervals from the non-syphilitic rabbits 7 to 14 weeks after the injection of tubercle bacilli into the skin presented much the same histological characteristics as those observed 5 to 6 weeks after infection with tubercle bacilli. Surface ulceration tended to become smaller with increasing age of the lesion and in nearly every instance was covered by regenerated

epithelium which occasionally grew downward. A single, large, sharply defined tubercle was noted, consisting essentially of epithelioid cells with some plasma cells and surrounded by connective tissue which gradually increased in density (Fig. 7). The epithelioid cells contained an eccentrically placed nucleus, poor in chromatin, and with a pale staining foamy cytoplasm. A sharply defined, rounded, paler staining zone was frequently noted in the cytoplasm immediately beneath the nucleus. In the deeper and papillary layers of the derma and in the subcutaneous tissue areas of granulation tissue rich in plasma cells were present. The vessel walls continued to increase in thickness. Small aggregations of plasma cells occurred throughout the tubercle and to a greater degree between the connective tissue fibers. Lymphocytes were relatively inconspicuous until 9 weeks after infection, when small aggregations were noted, most frequently at the margin of the tubercle.

Small areas of caseation, frequently infiltrated with dead or degenerating polymorphonuclear cells, were observed in the deeper part of the tubercle, while more extensive areas of caseation were noted in the papillary layer of the derma. Extensive softening of the caseous areas was noted about 7 weeks after infection and continued to extend throughout the period of observation.

Giant cells were observed with increasing frequency during this time. These were found most frequently in the papillary layer of the derma. Tubercle bacilli were again noted within mononuclear cells from the 7th to the 12th week following infection, while no tubercle bacilli were demonstrable 13 and 14 weeks after infection.

In the lesions removed from the syphilitic rabbits at weekly intervals from the 7th to the 14th week inclusive following the injection of bovine type tubercle bacilli into the skin, the surface ulceration was more extensive than among the non-syphilitic rabbits and no regeneration of the epidermis had taken place. The surface ulceration was separated from the underlying tissue by a line of demarcation consisting of polymorphonuclear cells, many of which had degenerated. Beneath the surface ulceration and throughout the derma there were aggregations of mononuclear cells with large, rounded or elliptical nuclei, rich in chromatin and surrounded by pale staining protoplasm. There were also small numbers of young epithelioid cells and plasma cells. These aggre-

gations were surrounded by tissue rich in young fibroblasts as well as by dense connective tissue.

Beginning 8 weeks after infection with the tubercle bacilli there was, in the syphilitic animals, an increasing number of mature epithelioid cells within the previously described aggregations of mononuclear cells. These epithelioid cells did not differ morphologically from those previously noted in the non-syphilitic rabbits. On the other hand, there was a marked difference in the distribution and arrangement of these cells. In contradistinction to the single large epithelioid tubercle noted in the non-syphilitic rabbits, among the syphilitic animals there were numerous epithelioid tubercles separated from one another by more or less dense bands of connective tissue. There was a marked increase of dense connective tissue in the deeper layers of the derma as well as in the subcutaneous tissue (Fig. 8). The walls of both small and large vessels were thickened but to no greater degree than among the non-syphilitic rabbits. Small areas of caseation, in some instances infiltrated with degenerated leukocytes, were noted. These areas of caseation, occurring within the nests or aggregations of epithelioid cells, became more numerous and more extensive, resulting in the confluence of these areas, with the formation of large, caseous masses, much greater in extent than among the non-syphilitic rabbits. Softening was not observed until 13 weeks after infection.

In these rabbits, during the period from 8-13 weeks after the infection, small collections of plasma cells, in some instances about blood vessels and between connective tissue fibers, as well as small collections of lymphocytes, were present. The number, arrangement and morphology of the giant cells were not conspicuously different in the two groups of animals during this period. Tubercle bacilli were not observed in the sections removed at weekly intervals from the 7th to the 10th week inclusive. An occasional cell containing some tubercle bacilli was seen in the lesions removed 11 weeks and later following infection with the tubercle bacilli.

SUMMARY, DISCUSSION AND CONCLUSIONS

These experiments indicate that syphilitic rabbits react in a different manner to the injection of living virulent tubercle bacilli than do similarly infected non-syphilitic rabbits. In the syphilitic

rabbits the local inflammatory reaction following the injection of tubercle bacilli into the skin was more intense in character, as evidenced by the greater degree of edema and cellular infiltration about the widely dilated vessels. As early as 3 hours after infection, and persisting throughout the period of observation, it was noted that in the syphilitic rabbits the lesions were multiple, focal in character and distributed about the capillaries, whereas in the non-syphilitic rabbits the lesion was single, diffuse in character and bore no relation to the vascular distribution. Histologically the lesions removed at intervals of 48 hours to 3 weeks following the injection of the tubercle bacilli into the syphilitic rabbits consisted of perivascular aggregations of large mononuclear cells, fibroblasts and newly formed capillaries. These lesions resembled in their histological characteristics the primary and secondary lesions of syphilis rather than the characteristic lesion of tuberculosis, although scrapings from more than 200 such lesions examined by means of dark-field have failed to show the presence of *Tr. pallidum*. Epithelioid cells, which were observed in both syphilitic and non-syphilitic rabbits about 2 weeks after infection, were fewer among the syphilitic rabbits and perivascular in their distribution, replacing the large mononuclear cells. On the other hand, among the non-syphilitic rabbits the epithelioid cells appeared in large numbers scattered diffusely throughout the tissue. In the syphilitic rabbits fibroblasts and newly formed capillaries made their appearance within 48 hours after infection with tubercle bacilli and increased rapidly in number, with the formation of dense bundles of connective tissue which separated the lesions, while in the sections removed from the non-syphilitic rabbits there was a paucity of fibroblast formation and an absence of capillaries.

There are considerable data (Bieling,¹² Hektoen,¹³ and Clark, Zellmer and Stone¹⁴) indicating that animals previously immunized to a specific antigen and whose blood serum after a lapse of time contains little or no specific antibody, will, when subsequently injected with an unrelated or remotely related antigen, respond with the production not only of antibodies against the last antigen but also with the production of antibodies against the initial antigen. The antibodies against the initially injected antigen appeared within 24 hours, whereas the antibodies against the second unrelated antigen appeared several weeks later. This revived produc-

tion of antibodies to the original antigen Bieling¹² has termed the "anamnestische Reaktion." That previous or concomitant disease may stimulate the production of antibodies has been noted by Schroeder,¹⁵ who found that rabbits suffering from spontaneous subcutaneous abscesses produced antisera with a higher titer than did normal rabbits. Similarly, Lewis and Loomis¹⁶ noted that antibody production in tuberculous guinea pigs greatly exceeded that obtained in non-tuberculous animals.

Much less observed data are available to indicate that this same type of "anamnestischem" mechanism is involved in the cellular reaction to an irritant. Tarnowsky¹⁷ and Greenbaum and Madden¹⁸ found that the application of a caustic to the skin of patients suffering from latent syphilis produced characteristic local syphilitic lesions. Klauder¹⁹ and others have shown that syphilitic lesions may occur at the site of trauma, although Klauder was unable to produce an interstitial keratitis in syphilitic rabbits by traumatizing the cornea. Moreover, Greenbaum and Madden could not demonstrate *Tr. pallidum* in local syphilitic lesions produced by trauma.

Our observations on the cellular response of syphilitic rabbits to the injection of virulent bovine type tubercle bacilli suggest that the reaction is of the "anamnestische" character and that the cells of the syphilitic rabbits are so modified that the introduction of an unrelated organism elicits a prompt inflammatory reaction characteristic of the initial syphilitic infection. The perivascular focal character of the lesion, the presence of large mononuclear cells and fibroblasts, and the formation of new vessels suggest syphilis; on the other hand the subsequent appearance of epithelioid cells and of caseation and softening are more characteristic of tuberculosis.

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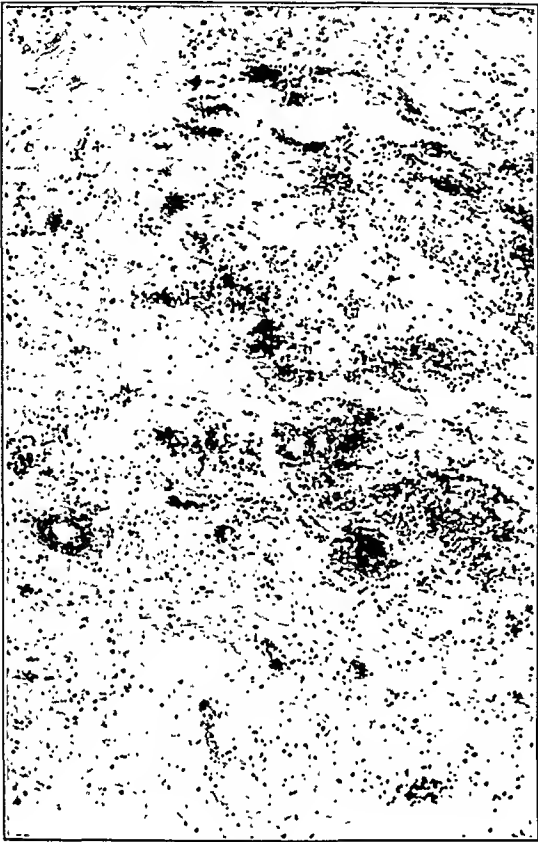
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DESCRIPTION OF PLATES

PLATE 35

- FIG. 1. Skin of syphilitic rabbit 3 hours after the injection of tubercle bacilli. Edema, aggregations of cells and perivascular infiltration. $\times 80$.
- FIG. 2. Skin of non-syphilitic rabbits 6 days after injection of tubercle bacilli. A sharply defined abscess. $\times 120$.
- FIG. 3. Skin of syphilitic rabbit 6 days after the injection of tubercle bacilli. Multiple foci of mononuclear cells. $\times 120$.
- FIG. 4. Skin of normal rabbit 6 weeks after injection of suspension of *Tr. pallidum*. Focal perivascular aggregation of mononuclear cells. $\times 400$.



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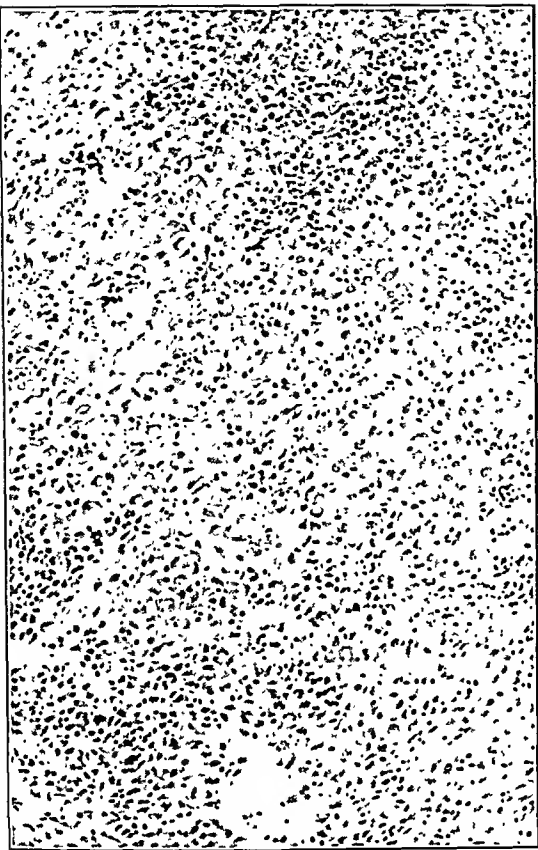
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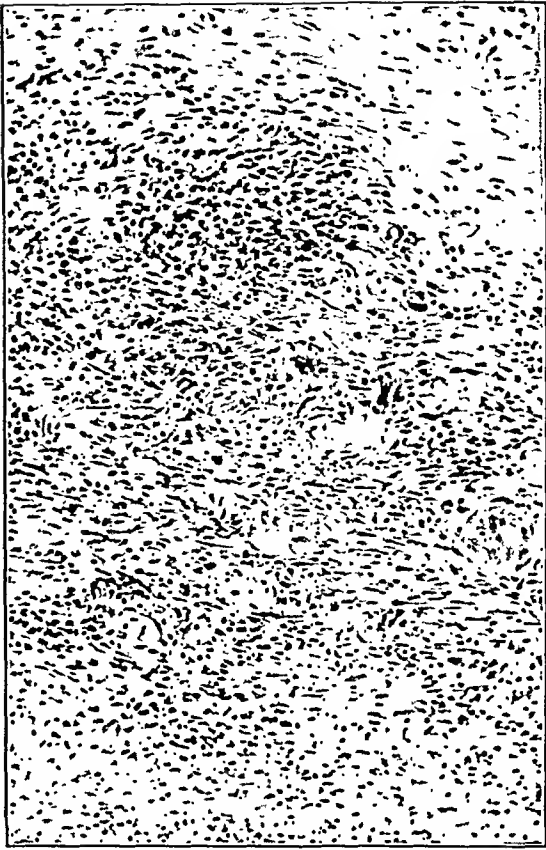
Effect of Syphilis on Tuberculous Lesions

PLATE 36

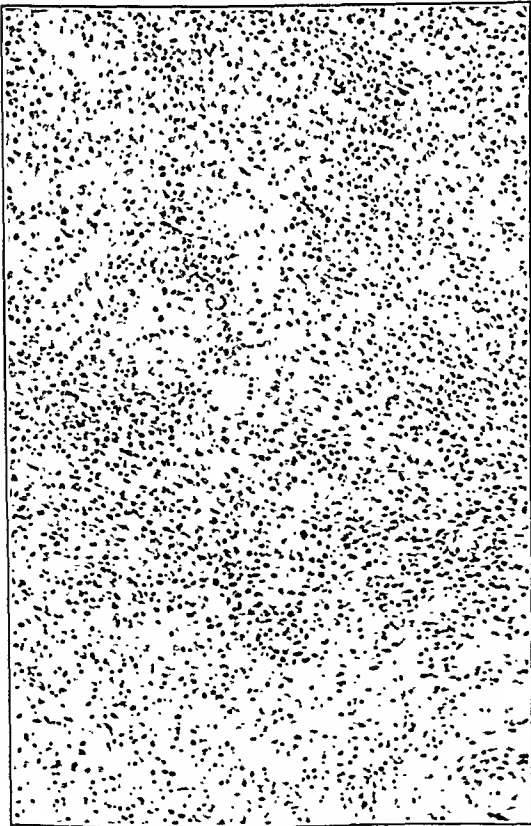
- FIG. 5. Skin of non-syphilitic rabbit 4 weeks after injection of tubercle bacilli. Diffuse infiltration with epithelioid cells. $\times 150$.
- FIG. 6. Skin of syphilitic rabbit 4 weeks after injection of tubercle bacilli. Granulation tissue rich in fibrous tissue and mononuclears. $\times 150$.
- FIG. 7. Skin of non-syphilitic rabbit 8 weeks after injection of tubercle bacilli. Diffuse infiltration with lymphocytes, plasma cells and epithelioid cells. $\times 120$.
- FIG. 8. Skin of syphilitic rabbit 8 weeks after injection of tubercle bacilli. Dense connective tissue replacement. $\times 120$.



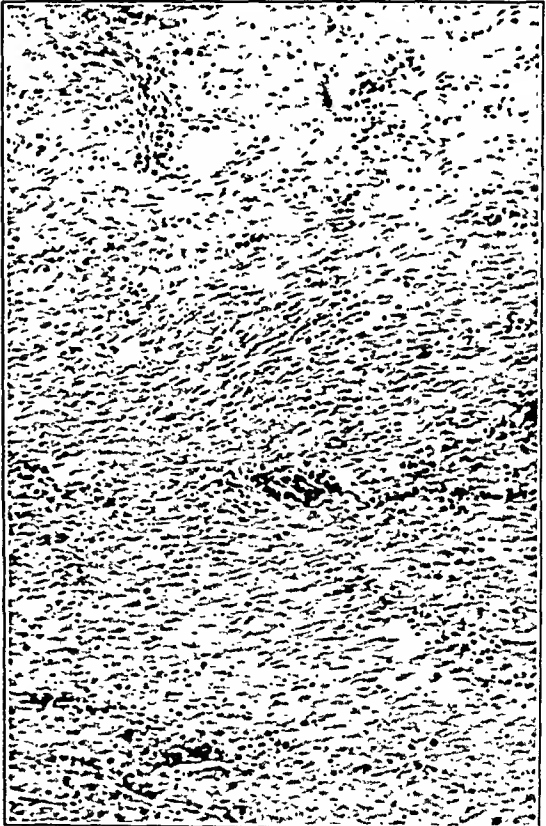
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SPONTANEOUS CARDIOVASCULAR DISEASE IN THE RAT *

I. LESIONS OF THE HEART

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Relatively little attention has been directed toward naturally developing cardiovascular disease in any species other than man. The contributions of Fox ¹ on natural disease in captive wild animals, and of Krause ² and Wolkoff ³ on the senile changes in the vascular system of domestic animals, serve as outstanding exceptions. Systematic observations of cardiovascular lesions in the common, small laboratory animals have been less often recorded. There are a few sporadic reports such as the description of spontaneous myocarditis in rabbits by Miller, ⁴ and of medial lesions in the aorta of this same species, originally noted by Ophüls ⁵ and by Miles. ⁶

Even less information is available concerning cardiac diseases to which the rat is naturally susceptible. Löwenthal ⁷ in 1931 was unable to collect any records of this type of disease in rodents other than those of inflammatory changes in the myocardium. McCay, Crowell and Maynard ⁸ in a study of the relation of growth to longevity mention that the hearts of old rats are constantly enlarged. A diligent search of the literature has failed to reveal any other reports dealing with this subject although the incidental description of such lesions in control rats used for other purposes may have escaped attention.

The elucidation of this field is of some practical significance. A comparison of spontaneous lesions in animals with those of man may be of value since differences in habits and environment as well as time factors related to life span may shed light on their etiology. It has sometimes been stated (Sherman ⁹) that the rat is rather similar to man in its omnivorous food habits and in most aspects of the chemistry of nutrition. It might not be unreasonable to suspect that if these factors play a rôle in the development of

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cardiovascular disease, this species should closely parallel man in this respect.

That distinct specie idiosyncrasies exist in the development of both spontaneous and experimentally produced cardiovascular disease seems fairly well established. Fox¹ describes variations in the types of spontaneous lesions to which different species are vulnerable. Information concerning spontaneous disease may be of importance in evaluating the results of a given experimental procedure. Cowdry and Scott¹⁰ have demonstrated that repeated doses of vitamin D in the monkey do not regularly lead to calcification of arteries, as in the rabbit and rat. Lesions developing in an animal on an experimental regimen are not necessarily the specific result of that procedure even if control animals of a similar age period fail to show them. The procedure may so debilitate and impair the general health of the animal as to precipitate the earlier initiation of lesions that might well have developed spontaneously later in life. The numerous, varied experimental methods that have led to medial degeneration of the rabbit's aorta lose much of their significance when it is appreciated that 87 per cent of all rabbits at 8 months of age exhibit similar lesions (Kesten¹¹). The importance of knowing the inherent disturbances and weaknesses of this system in measuring the effects of any experimental procedure is obvious.

For these reasons a systematic study of cardiac disease in a group of 487 rats was undertaken. The animals were derived from an inbred strain of pure albinos of Osborn-Mendel stock and maintained for more than 30 generations in the laboratories of Drs. H. C. Sherman and H. L. Campbell of the Chemistry department of Columbia University.* The influence of diet on longevity in this strain has been previously reported by these authors.¹² From the time of weaning the animals were fed adequate diets of known composition and maintained under constant laboratory conditions until death occurred spontaneously. The group includes 266 females whose average age at death was 746 days, and 221 males averaging 702 days in age at death. The youngest animal to die spontaneously was a female at 79 days, and the oldest also a female at 1124 days. The majority (79.2 per cent) survived longer than

* We wish to acknowledge our indebtedness to Dr. H. C. Sherman and Dr. H. L. Campbell for providing us with the opportunity of autopsying the animals on which this study is based.

600 days and 24 or 4.9 per cent (19 females and 5 males) were older than 1000 days.

The maximum life span of the rat is usually considered to be about 3 years or roughly $1/30$ th of that of man. At this ratio a period of 100 days in the rat is equivalent to 8.2 years in man. The average age at death in this group of rats would be equal to about 58 years of human life for the male and 61 years for the female. The average age at death for a similar group of humans would compare closely to these figures. The work of McKay, Crowell and Maynard⁸ would seem to indicate that the maximum life span in the rat is somewhat longer than 3 years, since they succeeded in sustaining life in 12 out of 106 animals for more than 1200 days. Their oldest rat survived 1421 days. The senile rats of the group reported here showed a high incidence of suppurative infections in the lungs, middle ears, brain and genito-urinary tract. If these had not occurred the survival periods would undoubtedly have been prolonged. Nevertheless, at autopsy even the human subject often shows evidence of infectious processes which may be unrelated to the principal underlying disease. Thus, lobular pneumonia of varying extent is encountered in more than 50 per cent of all human autopsies. It would perhaps be futile to attempt a more accurate comparison of life spans from our present knowledge.

Each animal in the group was subjected to a detailed postmortem examination. Tissues were fixed in Zenker's fluid without addition of acetic acid, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Special connective and elastic tissue stains were employed when required. One complete sagittal anteroposterior section through the entire heart was prepared for microscopic study. This included routinely the left ventricle, interventricular septum, right ventricle, portions of the valve leaflets, auricles and ascending aorta. The following presentation is a description of the pathological findings encountered. The more common lesions are grouped and classified. The incidence and influence of sex and age were determined. The relations of the lesions to each other or to extracardiac changes was explored whenever a possible connection existed. The composition of the diets was analyzed to see if any favored or inhibited the development of these lesions. Reference to diet will be made only when such an influence is indicated.

ENDOCARDIUM

Intracardiac Thrombosis: In 31 animals (6.4 per cent) thrombi were found in one or more chambers of the heart. 17 of these were females and 14 males. Fairly old rats were particularly susceptible. The average age of the males with cardiac thrombosis at death was 799 days, compared with 702 days for the entire group, and of the females 905 days compared to 746 days for the entire group. In fact, 7 or 29.2 per cent of the 24 animals surviving more than 1000 days showed the lesion. The left auricle was by far the most common site, being involved 20 times. The right auricle was next with 7 cases. Two parietal thrombi had deposited on the wall of the left ventricle overlying areas of myocardial and endocardial fibrosis even though this chamber lacks columnae carneae to form favoring endocardial recesses. Two other thrombi were found loose and detached; one caught in the orifice of the mitral valve, presumably after having broken free from its origin in the left auricle, and one occluding the aortic valve ring.

Only the larger thrombi completely filling the left auricle were recognized grossly. They could be distinguished by their firm inelastic consistence, grayish red color, and by the marked auricular dilatation. The majority of the thrombi in the left auricle were of this type. They apparently obstructed blood flow completely. Usually the thrombi were of recent formation, microscopically showing little or no evidence of organization. They were composed of alternating columns of massed platelets, fragmented leukocytes and intervening deposits of other blood constituents. They were as a rule only loosely attached to the underlying endocardium. In some instances only smaller parietal thrombi were found, most often in pockets of the auricular appendages between musculi pectinati.

Extracardiac infectious processes were neither more severe nor more numerous in this group than in the other animals. Tumor growths were not found in any of the 31 rats. Factors favoring the development of thrombi were usually present in the heart itself. 23 of the animals showed some degree of sclerosis of the coronary arteries, of which 15 were either moderate or severe in degree. 25 showed evidence of myocardial fibrosis, of which 23 were either moderate or severe. As will appear shortly, the incidence of these

two lesions was just as high in animals of a comparable age as in this particular group. Moreover, myocardial fibrosis was rarely encountered in the walls of the auricles. It is therefore difficult to evaluate the rôle of either of these two changes in favoring the formation of intracardiac thrombi.

There was, however, still a third lesion, apparently a chronic inflammation involving chiefly the endocardium of the left auricle, which appeared to play a definite rôle in the precipitation of thrombi in at least some instances by damaging the surface endothelium. Such changes, designated as chronic auriculitis, were found in 8 of the 20 showing thrombi in the left auricle.

Chronic Auriculitis: This lesion was recognized on microscopic examination in 18 (3.7 per cent) rats, 10 females and 8 males. The left auricle alone was involved in 15 cases, twice in conjunction with slight alterations of the left ventricular endocardium and once with minor changes in the right auricle. The lesion attacked a fairly old age group. The average age at death of the females was 819 days, and of the males 758 days. Both sclerosis of the coronary arteries and myocardial fibrosis were extremely common in these animals, but there were 3 in which both of these lesions were entirely absent, so that it can hardly be considered as a secondary extension or complication.

The lesion usually involved the entire endocardial surface of the auricle without extending into the appendage or onto the auricular aspect of the mitral valve leaflets. Microscopically the essential finding was a marked thickening of the endocardial layer. As in man, the left auricular endocardium is normally thicker than the right, although smooth muscle cells cannot be identified. With the development of this lesion, the endocardium increased in thickness 4 to 6 times. The picture varied considerably, depending probably on the stage of development and severity of the process. The endothelial cell lining was usually intact although swollen, basophilic and prominent. Occasionally defects were present and fibrin thrombi were precipitated. The entire endocardium was infiltrated with a variable number of cells. Large numbers of mononuclear wandering cells were frequently encountered, but occasionally lymphocytes, plasma cells or even polymorphonuclear leukocytes predominated. More often there was a mixture of cells including proliferating fibroblasts. The thickening was due in part to the

cellular infiltration but also to an irregular production of connective tissue fibers so that the surface frequently became uneven. In some instances connective tissue proliferation was the conspicuous finding, presumably in arrested or relatively inactive cases. The endocardium did not become vascularized.

The lesion is at once reminiscent of the auriculitis associated with rheumatic heart disease in man, both because of its location and because of its microscopic detail. However, palisading of cells against swollen collagen bands, irregular pyknotic nuclear forms and well defined Aschoff nodules did not occur. In addition, the valve leaflets and perivascular connective tissue of the myocardium were not involved by the specific lesions of rheumatic fever. For these reasons it is impossible to associate it with rheumatic infection. The etiology of the process is completely shrouded although its microscopic characteristics indicate that it is inflammatory in nature. Bacteria were not demonstrable.

Bacterial Endocarditis: The heart valves of the rat were occasionally the seat of bacterial infection which reproduced the local picture of acute bacterial endocarditis in man. 15 such cases (3.08 per cent) are included in this series, 9 in males and 6 in females. The age of these rats at death was widely dispersed, the youngest animal being 138 days and the oldest 1072 days. The average age for the group was well below that of the entire series, being 650 days in the male and 566 days in the female. It is interesting that the incidence in which the individual valves are involved compares closely to that in man.

The mitral valve was attacked 11 times, the tricuspid once, the aortic once, and all three mitral and aortic valves and left auricle once. In one instance the infectious process appeared to originate in the endocardium of the right ventricle, although multiple sections might have revealed a focus in the valve. The microscopic picture was somewhat variable. The less severe lesions centered about the distal ends of the leaflets. More often the entire leaflet was involved, its substance eroded so that only remnants of the valve could be made out. Heavy cellular infiltrations of polymorphonuclear leukocytes and other inflammatory cells occurred regularly. Occasionally abortive attempts at repair were evidenced by accumulations of large mononuclear cells and fibroblastic activity. The damaged surfaces of the valves were covered by bulky

vegetations composed of precipitated fibrin, large bacterial colonies and cellular débris. Often the vegetation filled the entire valve orifice and protruded into the adjacent chambers.

The chief extravalvular finding was metastatic abscesses in the myocardium and kidney, and less often at other sites. The spleen was usually enlarged but not pultaceous. Inasmuch as enlargement of the spleen usually accompanies other infections coexistent in these rats, it is difficult to attribute it solely to the blood stream infection. Gross embolic manifestations were conspicuously absent. In several instances bacteria had gained a foothold in the walls of some of the arteries and had led to arteritis and thrombosis, but infarcts of the spleen and kidney were not seen.

It was not usually possible to establish the exact portal of entry of the bacterial agent. This was due to the presence of too many possible sources rather than too few. Most of the animals had infected ulcerations of the plantar surfaces of the extremities and tail, any one of which might have served as the initial point of infection. In addition, 12 had bilateral suppuration of the auditory bullae, 5 bronchiectatic cavities and abscesses in the lungs, and 2 extensive suppurative endometritis. It must be admitted that infections of the same type and severity were just as frequent in the animals with normal heart valves.

Bacterial cultures were not made so that no accurate information concerning the causative agents can be furnished. Bacteria were, however, readily demonstrable in the vegetations. In most instances they appeared to be Gram-positive cocci, but in two, Gram-negative rods were identified. Although usually the valve leaflets were too completely destroyed to make any positive statements as to their condition previous to infection, there is no reason for assuming that preceding damage existed. Unaffected leaflets in the same hearts showed little or no evidence of alteration.

The normal valve leaflets of the rat are delicate fibrous acellular structures having a little centrally placed elastic tissue and regularly failing to reveal the presence of blood channels. At the base of the aortic valve, cartilaginous plates are embedded so often as to be considered a normal component of the annulus. With advancing age the valves underwent only slight changes. The annulus frequently became larger and often directly continuous with myocardial scars at the base of the heart. Rarely small masses of

calcium were deposited either at the base of the atrioventricular leaflets or in the cartilage of the aortic valve leaflets. The substance of the valve became more solid and compact but remained avascular. Just proximal to the distal end of the mitral valve leaflets on the auricular aspect, and coinciding with what must have been the line of closure, a mound-like eminence of loose edematous connective tissue usually developed. Because of the apparent avascularity of the leaflets, the assumption must be made that bacterial infection occurs as a surface implantation possibly at some minutely damage point on the line of closure. It would probably require technically difficult injection experiments to rule out beyond question the existence of valvular blood channels. Much of the theory that has been expounded to account for the greater frequency of involvement of the mitral valve in man has been based on the more common vascularization of this valve. In the light of the present findings this explanation loses at least some of its validity.

It should be pointed out that in every instance the lesion was that of an acute spreading bacterial infection and never was the picture of subacute bacterial endocarditis in man reproduced. The valvular lesions were too acute and destructive and embolic lesions when they involved the renal glomeruli were frankly purulent.

"Chronic Endocarditis": The usual appearance of the heart valves has already been described. In addition to the well defined cases of bacterial endocarditis there were 15 cases in which the heart valves showed minor deviations from the normal. For want of a better term these have been designated as "chronic endocarditis," although the changes were perhaps too minute to be so classified. Never was there obvious deformity or insufficiency of any valve. In 7 the lesions consisted essentially of a mild but definite cellular infiltration of large mononuclear cells into the substance of the mitral valve most conspicuously near the distal end and on the auricular aspect. This was sometimes associated with slight edema of the connective tissue and heaping up of the overlying endothelium. In 4 more, the mitral valve showed, in addition, slight defects in its endothelial surface over which small quantities of fibrin or fibrin-like material were attached. In no instance was there true verruca formation. The tricuspid valve shared these lesions in 3 cases; the mitral and aortic valves were involved together in 1 case. The exact interpretation of these

changes is in doubt. Bacteria were not demonstrable. Possibly they merely represented the results of slightly excessive trauma. The valvular lesions of human rheumatic infection were never very closely simulated.

MYOCARDIUM

Fibrosis of Myocardium: One of the most common findings encountered was scarring of the heart muscle. 292 or 59.9 per cent of the animals showed some degree of myocardial fibrosis. Males (65.6 per cent) were more often involved than females (55.3 per cent). The sex difference was more definite when the degree of fibrosis was compared, as in Table I. Here it can be seen that 14.5 per cent of the males and only 6.8 per cent of the females showed severe fibrosis — a ratio of more than 2:1. Fibrosis of the myocardium also occurs more often and severely in the human male than in the female. In the case of the rat, differences in environmental factors cannot be held accountable.

The lesion showed a definite relation to age, tending to involve older animals. The average age at death increased with the severity of the fibrosis in both males and females. In view of the fact that the female rat has a distinct advantage in longevity over the male, the sex difference noted above is even more striking. The average age of the 32 males exhibiting marked fibrosis at death was 805 days and of the 18 females 896 days. The age factor in relation to fibrosis is more apparent from the figures in Table II where the animals are grouped in 100 day intervals. Only 2 males between 500 to 600 days old showed severe fibrosis and none at a younger age. It may be seen that the milder degrees of scarring occurred more often in younger animals, but that animals less than 400 days old almost always had intact myocardia. With advancing age groups, the lesion became more frequent and severe and, in general, the males showed earlier and greater involvement than the female. The impression is gained therefore that fibrosis of the myocardium in the rat depended to a large extent upon age and sex. It appeared to be a progressive lesion having its inception about the 400th day of life and becoming more marked throughout the rest of life. Nevertheless, there was a great deal of individual variation and a good proportion escaped the lesion entirely. Close analogies to human arteriosclerotic heart disease might be drawn,

TABLE I
Incidence of Myocardial Fibrosis

	No fibrosis		Slight fibrosis		Moderate fibrosis		Marked fibrosis		Total with fibrosis	
	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent
Males	76	34.4	62	28.1	51	23.0	32	14.5	145	65.6
Females	119	44.7	63	23.7	66	24.8	18	6.8	147	55.3
Total	195	40.1	125	25.7	117	24.0	50	10.2	292	59.9

TABLE II
Age Distribution of Myocardial Fibrosis

Age	Sex	No fibrosis		Slight fibrosis		Moderate fibrosis		Marked fibrosis	
		No. of animals	Per cent *	No. of animals	Per cent *	No. of animals	Per cent *	No. of animals	Per cent *
days	Male	8	88.9	1	11.1	0	—	0	—
	Female	21	95.5	0	—	1	4.5	0	—
400-500	Male	10	55.6	7	38.9	1	5.6	0	—
	Female	11	100.0	0	—	0	—	0	—
500-600	Male	14	51.9	9	33.3	2	7.4	2	7.4
	Female	9	64.3	4	28.6	1	7.1	0	—
600-700	Male	20	40.0	12	24.0	14	28.0	4	8.0
	Female	15	48.4	11	35.5	4	12.9	1	3.2
700-800	Male	12	21.8	20	36.3	16	29.1	7	12.7
	Female	26	38.8	17	25.4	18	26.9	6	9.0
800-900	Male	9	21.9	10	24.4	7	17.1	15	36.6
	Female	24	36.4	18	27.3	22	33.3	2	3.0
900-1000	Male	2	12.5	3	18.7	9	56.3	2	12.5
	Female	13	36.1	11	30.6	10	27.8	2	5.5
1000-1125	Male	1	20.0	0	—	2	40.0	2	40.0
	Female	0	—	2	10.5	10	52.6	7	36.9

* Per cent of all rats in the same 100 day interval.

not only in chronological relation to life span, but in frequency and sex distribution.

The fibrosis was entirely a microscopic finding. The distribution of the scarring varied somewhat but there were certain vulnerable areas. The left ventricle and interventricular septum were by far the most common sites. The earliest scars were found at the base sometimes extending as tenuous bands from the annulus of the mitral valve or at the tip of the left ventricle. In the latter region the muscle layer is normally quite thin and in advanced cases the entire muscle from epicardium to endocardium was replaced by dense connective tissue. Frequently scars seemed to center about and extend from the adventitia of the larger coronary arteries as they coursed through the muscle layer. At times they were haphazardly distributed. The right ventricle was almost never involved save only at its very base. The auricular muscle remained intact.

For the most part the scarring was partial and rather diffuse, isolated groups of atrophic or hypertrophic muscle bundles being intermingled. Not infrequently fibrous tissue was very finely distributed but widespread, appearing as a generalized increase in loose interstitial connective tissue. Rarely large continuous masses of partly hyalinized, completely acellular connective tissue devoid of muscle fibers had formed. These most closely resembled healed infarcts. True fresh or partly healed infarcts were never found and it seems very doubtful that any of the fibrosis was preceded by necrosis of muscle. Blood pigment and mononuclear cells were usually lacking. The absence of cellular reaction makes it seem most probable that the fibrosis was associated with a slow atrophy of the muscle either due to impaired blood supply or to intrinsic degeneration of the muscle tissue itself. In a few instances cellular reaction was very striking, a variety of leukocytes participating. In these, the evidences of inflammation were so apparent that the lesion might well be classified as chronic myocarditis. This finding occurred so seldom that it is difficult to believe that the more usual type of scarring was the result of a previous inflammatory process which had become arrested. Moreover, the earliest lesions showed no tendency to exhibit an inflammatory character. The specific localizations of the scarring in certain parts of the heart also argues against an infectious background.

As will appear shortly, alterations of the coronary arteries, which would lead to loss of elasticity and hardening if not to complete closure, frequently accompanied the myocardial damage. The weight of the evidence at hand suggests that a process is involved somewhat analogous to arteriosclerotic heart disease in man, namely that with advancing age there is commonly a progressive sclerosis of the coronary arteries interfering with the nutrition of the myocardium and resulting in atrophy and fibrosis. In the rat the vascular lesion is essentially a medial one and never leads to occlusion or thrombosis so that the acute lesions of myocardial infarction do not develop.

Sclerosis of Coronary Arteries: As previously noted, showing close coincidence with fibrosis of the myocardium, the coronary arteries frequently developed changes that consisted essentially of a loss of smooth muscle and a replacement of the media by fibrous tissue. The intima remained thin, the lumen patent, although often reduced to a slit, and the internal elastic lamella although straightened retained its unity. The earliest visible change was a reduction and irregular distribution of smooth muscle nuclei of the media. Some of the muscle cells were hypertrophied and on cross section appeared strikingly vacuolated. Accessory bunches of smooth muscle cells forming imperfect new coats occasionally developed in the adventitia or beneath the intima to give the vessel irregular contours, make it thicker than normal, and encroach somewhat upon the lumen. The adventitia became thickened and densely fibrous. Eventually connective tissue replaced the smooth muscle and this often became hyalinized. Calcification of the media occurred rather infrequently but was noted in 17 cases. It was usually rather scanty, consisting of small deposits in and beneath the elastic lamella. In one instance the calcification was extensive enough to form curved plates within the media, incompletely encircling the entire circumference of the right coronary artery just beyond its point of origin. The intima remained delicate, consisting of a single layer of endothelium closely approximating the subjacent elastic fiber. Lipoid deposits in the intima, similar to atherosclerosis of man, were not observed.

The coronary arteries of the rat's heart are two in number and arise from the sinuses of Valsalva at points similar to the origin of the coronary arteries in man. They also follow the usual distribu-

tion of the human vessels, the right being slightly the larger and supplying portions of the left ventricle posteriorly and the interventricular septum. In the atrioventricular sulci the coronary arteries lie in the subepicardium sometimes embedded in a slight amount of adipose tissue. Subepicardial fat in the rat's heart is always scanty, even in well nourished animals, and is confined to the base of the ventricles. All the branches of the main coronary arteries equivalent to the descending branches in man are definitely intramuscular, although the coronary veins course superficially. The microscopic appearance of the normal coronary artery shows no striking peculiarities. They possess a single internally placed, wavy elastic lamella. Both vessels appeared to be involved with equal frequency and severity by the sclerotic process. The most profound changes were found in the main arteries just beyond their points of origin but the lesion extended well into the intramuscular branches. One exception was a small artery rather constant in position near the base of the anterior leaflet of the mitral valve which often showed marked fibrosis when all other vessels were quite normal.

From Table III it may be seen that, as in fibrosis of the myocardium, the male is more often and severely affected than the female. The incidence of severe coronary sclerosis is again almost 2:1 in favor of the male. Similarly it may be seen that the total incidence of each grade of involvement closely parallels that of myocardial fibrosis by comparing the figures in Table I. 57.7 per cent of all the animals showed some degree of coronary arteriosclerosis, whereas 59.9 per cent showed some myocardial fibrosis.

Table IV shows the age distribution at 100 day intervals and discloses that age plays an analogous rôle in the development of the arterial lesion, as it did in myocardial fibrosis. There is this exception however. The milder degrees of arterial change not infrequently made their appearance in the first 400 days of life. This might be taken as evidence that the vascular lesion antedated the muscular scarring and if any cause and effect relation between the two lesions existed, the arteriosclerosis was responsible for the subsequent myocardial degeneration.

Although the two lesions were closely linked as regards frequency, age and sex distribution, the two did not always parallel each other in individual cases. For example, 16 animals that

TABLE III
Incidence of Sclerosis of Coronary Arteries

	No sclerosis		Slight sclerosis		Moderate sclerosis		Marked sclerosis		Total with sclerosis	
	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent
Males	86	38.9	51	23.1	59	26.7	25	11.3	135	61.1
Females	120	45.1	72	27.1	58	21.8	16	6.0	146	54.9
Total	206	42.3	123	25.3	117	24.0	41	8.4	281	57.7

TABLE IV
Age Distribution of Sclerosis of Coronary Arteries

Age	No sclerosis		Slight sclerosis		Moderate sclerosis		Marked sclerosis	
	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent
<i>days</i>								
0-400	25	80.6	4	12.9	2	6.5	0	—
400-500	22	75.9	3	10.3	4	13.8	0	—
500-600	23	56.1	13	31.7	5	12.2	0	—
600-700	41	50.6	20	24.7	13	16.1	7	8.6
700-800	46	37.7	35	28.7	28	23.0	13	10.6
800-900	30	28.0	27	25.2	37	34.6	13	12.2
900-1000	19	38.5	11	21.2	17	32.7	5	9.6
1000-1125	0	—	10	41.7	11	45.8	3	12.5

exhibited moderate or marked fibrosis of the myocardium revealed apparently normal coronary arteries. On the other hand, 10 animals showing moderate or severe sclerosis of the coronary arteries had intact myocardia. It is likely that a more extensive microscopic examination would have yielded a closer correlation in these exceptional cases. Reference to Table V will show that in general there is a fairly close correspondence between the two lesions. 77.5 per cent of the animals showing no myocardial fibrosis had normal coronary arteries and 84 per cent with either moderate or severe fibrosis also had either moderate or severe sclerosis of the coronary arteries. In arteriosclerotic heart disease in man, the severity of the coronary artery lesion usually parallels the degree of myocardial damage but individual cases show just as marked discrepancies as do the two lesions in the rat.

Coincidental extracardiac lesions were analyzed to see if they played a rôle in the development of these lesions but no such relationship was discovered. One lesion of interest in this connection is the pulmonary one consisting of a combination of bronchiectasis and lung abscess and occurring in varying degree in about 75 per cent of the animals. Both by reason of its high incidence and because of the effects of a badly impeded pulmonary circulation, it is conceivable that this lesion might have been a factor in producing cardiac changes. However, distinct hypertrophy of the right ventricle was lacking. Moreover, 26 per cent of the animals showing little or no bronchiectasis had moderate or severe cardiac lesions. On the other hand, 31 per cent showing severe or moderate bronchiectasis had little or no cardiac damage, whereas only 19 per cent with equally severe bronchiectasis exhibited severe or moderate cardiac lesions. The implication of the latter finding is that animals with marked pulmonary lesions may have succumbed before reaching the age in which severe lesions of the heart were prevalent. Furthermore, the pulmonary lesions did not progressively increase in frequency with advancing age so uniformly as the cardiac ones. For these several reasons it seems quite unlikely that the cardiac damage resulted secondarily from impaired pulmonary circulation.

Cardiac Hypertrophy: The size of the heart of the adult rat at spontaneous death varied considerably and was dependent largely on the body size of the animal and the degree of dilatation. Since

TABLE V
Relation of Myocardial Fibrosis to Sclerosis of Coronary Arteries

	No sclerosis		Slight sclerosis		Moderate sclerosis		Marked sclerosis	
	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent
No fibrosis	151	77.5	34	17.5	8	4.1	2	1.0
Slight fibrosis	39	31.2	53	42.4	26	20.8	7	5.6
Moderate fibrosis	13	11.1	31	26.5	63	53.9	10	8.5
Marked fibrosis	3	6.0	5	10.0	20	40.0	22	44.0

the weight of the animal was influenced by the state of nutrition and the amount of postmortem dehydration, the ratio of body weight to heart weight was not a reliable criterion of hypertrophy. The weight of the heart was not recorded and estimations of hypertrophy were based on total size, ventricle wall thickness and the microscopic diameters of the individual fibers. It is admitted that these criteria are largely subjective and liable to error. 173 rats, 86 males and 87 females, were considered to have definite hypertrophy of the heart in some degree, an incidence of 35.5 per cent. Although the sexes were about equally involved, the enlargement in the male was often more striking. Hypertrophy was rarely detectable before 500 days of age and became more frequent with advancing age thereafter. The left ventricle was the chief site, so that this chamber often occupied a disproportionately large part of the entire organ. The increase in muscle was usually associated with some degree of dilatation.

Microscopically the muscle bundles were increased in diameter. This was more striking at the base of the left ventricle rather than at the apex, and often hypertrophic fibers were unevenly distributed, leaving many groups of muscle bundles unchanged. The structure of the hypertrophic fiber was essentially unaltered. The nuclei were not strikingly enlarged but were irregular and hyperchromatic. The interfibrillar substance was not increased.

Many of this group showed a coincidental myocardial fibrosis and changes in the coronary arteries. This is not surprising when it is recalled that similar age groups are involved. The total incidence of hypertrophy was much lower than that of the other two lesions, so that it was not an inevitable accompaniment of the latter. In fact, many of the most severely fibrotic hearts were quite small. 43 per cent of the animals with no hypertrophy did have some degree of fibrosis. Hypertrophy without fibrosis was seen in only 14, about 8 per cent of all those without fibrosis. The inference may be drawn that hypertrophy often accompanied fibrosis and coronary arteriosclerosis but was by no means a constant concomitant. Hypertrophy in the absence of other myocardial changes, although uncommon, also occurred. As in man, enlargement of the heart not infrequently accompanied chronic renal disease. 24 per cent of the hypertrophy group also had relatively severe renal lesions.

Infrequent Myocardial Lesions: Suppurative infections were extremely common in the entire series. The most usual sites were the auditory bullae, lungs, uterus, cranial cavity, kidneys, prostate and liver. In a few instances bacteria had gained entrance into the blood stream and given rise to metastatic foci. The heart was one of the most common sites for such secondary abscesses, being involved 17 times. The abscesses were usually small, fresh, well demarcated, often multiple and without an encapsulating membrane. They consisted of disintegrating polymorphonuclear leukocytes and centrally placed clumps of bacteria.

Aside from these definite foci of infection, it was not unusual to find a few scattered cells infiltrated into the interstitium and often in the adventitia of blood vessels. Occasionally these were grouped together in submiliary collections so as to resemble somewhat the appearance of Aschoff nodules, although in no instance was the reproduction very exact. Such lesions did not occur in conjunction with endocardial or pericardial changes. These cellular aggregates exhibited much individual variation. In 21 cases the resemblance to human Aschoff bodies was fairly close. They were apt to occur in hearts that were considerably scarred but could also be found in otherwise normal areas. Occasionally the cells in them were large basophilic and had hyperchromatic, heavily rimmed nuclei with prominent nucleoli. Swollen fragmented collagen bundles were usually lacking and never very definite.

The series includes 17 cases of leukemia in which infiltrations of leukemic cells into various organs were common. The heart was infrequently invaded but twice there were small collections of leukemic cells penetrating the interstitial and subendocardial tissue.

Relatively few malignant growths were observed in the present series and most of these were of connective tissue origin rather than epithelial. Only one, an osteosarcoma of undetermined origin had metastasized to the heart.

PERICARDIUM

Acute Suppurative Pericarditis: The pericardial sac was infected with bacteria 11 times, an incidence of 2.3 per cent. Five times the resultant inflammation was confined to small areas, usually at the base of the heart over the auricular epicardium, but in the others the entire surface was covered by thick layers of fibrinopurulent

exudate. Bacteria were readily demonstrable. Granulation tissue was seen at times in the deeper layers of the exudate. In 9 of these 11 there was extensive pulmonary and pleural infection, and in several direct extension to the pericardium could be traced. The remaining 2 had suppurative lesions elsewhere which might have served as an initial source. The underlying myocardium and the endocardium were unaltered.

Acute pericarditis in rodents caused by infection with a streptothrix has been described by Berberich and Nussbaum¹³ in hemorrhagic septicemia, and with a diplococcus by Seifried.¹⁴ The pericardial infection was the result of extension from pulmonary foci.

Chronic Pericarditis: The pericardium of the rat appears to be subject to a specific inflammatory process which because of its distinctive histological appearance seems to be a disease entity. The picture was that of a low grade, persistent inflammation attended by the infiltration of lymphocytes, plasma cells, large mononuclears and the local proliferation of mesothelial cells and fibroblasts. The epicardium was thickened and densely cellular. The overlying mesothelium was swollen, basophilic, heaped up in villus protrusions and even stratified. Often the infiltrating cells were lined up in palisade formation parallel to the surface and lodged against long acellular bands of eosinophilic collagen. Such palisades were often multiple. In more severe cases the surface mesothelium was disrupted and minute quantities of a fibrinoid substance were deposited in the exposed surface. Occasionally eosinophils were fairly numerous but neutrophilic polymorphonuclear leukocytes were always infrequent.

The process was not uniformly distributed and was usually most advanced in crevices and sulci about the auricular appendages or in the atrioventricular groove. Less often it involved the entire epicardium, but unevenly, with mound-like eminences irregularly scattered. The normal parietal pericardium was apparently so delicate as to be invariably ruptured on removing the chest plate. At any rate it was not often detected. Pericardial adhesions, which from the nature of the lesion might be reasonably expected, were not encountered. Grossly the epicardial surface was described as smooth but milky gray and translucent.

The lesion was not confined to the pericardium but frequently involved both pleural cavities and the peritoneum. In the latter

it was most often seen over the capsules of the spleen and liver, around the pancreas, mesentery and posterior peritoneal surface. The microscopic appearance was always essentially the same. In neither pleura nor peritoneum did obliterative adhesions form. Aside from the serous surfaces, no other tissues were involved. The process may be considered to be essentially a polyserositis.

29 such cases were recognized, an incidence of 6 per cent. In 13 all three serous cavities were involved and in the remainder the pericardium alone was attacked. One striking feature was its decided preponderance in female rats, there being only 5 males to 24 females in the group. The lesion was found in comparatively old rats. The age at death of the females was 878 days and of the males 735 days.

The etiology of this process is completely obscure. Microorganisms were not demonstrated, but cultures were not taken. Intracellular inclusion bodies were not encountered. It bore no definite relation to any other disease process. Suppurative infections, although common, were not more so than in the other animals. Three of the rats had inflammatory changes in the left auricular endocardium previously designated as auriculitis. Since both lesions were relatively infrequent (6 per cent and 3.7 per cent), this association is perhaps higher than might be expected. Myocardial fibrosis and cardiac hypertrophy were not more marked than in animals of similar age periods. Because of its distribution and chronicity, one is reminded of Pick's disease in humans, but arrested cases with calcification, hyalinization and obliterating adhesions were never observed. Occasionally several cubic centimeters of clear pale fluid were contained within the peritoneal cavity, but larger accumulations were lacking.

A similar disease, possibly etiologically related, has been described in guinea pigs by Steinmetz and Lerche¹⁵ and more recently by Roth.¹⁶ A Gram-labile bacillus was recovered from the lesions but an etiological relationship has not been definitely established. The guinea pig disease differs from that in the rat in that it is acute and fatal.

SUMMARY

The pathological manifestations of cardiac disease in a group of 487 inbred albino rats maintained on adequate diets and under

constant laboratory conditions over their entire life span are described. The animals were not subjected to experimental manipulation and the disorders encountered must be considered to have evolved from spontaneous causes and under natural circumstances. The rat's heart proved to be quite susceptible to a variety of disease processes, some of which are distinctive and peculiar to this species, while others have their counterpart in man. A simple tabulation of each type of lesions described and its incidence follows:

Endocardium

Intracardiac thrombosis	6.4%
Chronic auriculitis	3.7%
Acute bacterial endocarditis	3.4%
"Chronic endocarditis" (without valvular insufficiency)	3.4%

Myocardium

Fibrosis of myocardium	59.9%
Sclerosis of coronary arteries	57.7%
Sclerosis of coronary arteries with calcification	3.7%
Hypertrophy of myocardium	35.5%
Abscess of myocardium	3.7%
"Interstitial myocarditis"	4.3%
Leukemic infiltration	0.4%
Secondary osteosarcoma	0.2%

Pericardium

Acute suppurative pericarditis	2.3%
Chronic pericarditis	6.0%

Both the lesions classified as chronic auriculitis and chronic pericarditis are apparently peculiar to the rat, although the latter lesion may be related to a similar condition occurring in guinea pigs. They consist essentially of long-standing, low grade inflammatory changes. In neither case was the responsible etiological agent identified. The pericardial lesion appears to be merely one expression of a generalized disturbance of the serous surfaces.

All the other processes described resemble in part at least those recognized in man. The acute bacterial infections certainly differed in no essential manner. Intracardiac thrombosis, chiefly of the left auricle, is somewhat similar to the auricular appendage thrombosis in the diseased human heart. In the rat, however, the thrombus usually filled the entire auricle and occurred as a terminal event, especially in senile animals. Myocardial fibrosis of the left

ventricle is common to both species but in the rat it is less obviously the result of reduced arterial circulation. Changes in the coronary arteries are common and do parallel the myocardial lesions but they never lead to complete or even marked occlusion.

With the exception of the infectious processes, almost all of the changes described make their appearance late in the 2nd year of life and do not attain their maximum incidence until well into the 3rd year. These periods correspond roughly to late middle age and early senescence. Many of the human cardiac conditions have a similar age distribution. In both species the male is somewhat more susceptible to this type of disease than the female.

Notable in the rat by their absence are the intimal atheromas of human coronary artery disease, evidences of myocardial infarction, chronic valvular deformities and rheumatic infection. Slight, apparently non-specific inflammatory changes of the mitral valve and perivascular tissue of the myocardium, which might be erroneously construed if encountered in experimental animals, do occur but are relatively rare.

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DESCRIPTION OF PLATES

PLATE 37

- FIG. 1. Acute bacterial endocarditis and acute suppurative pericarditis. The arrow indicates a ball-like vegetation on the mitral valve leaflets. The epicardium is everywhere coated by a thick layer of fibrinopurulent exudate.
- FIG. 2. Cardiac hypertrophy. The variation in size of the heart at death in 2 old rats is indicated. The one on the left is considerably hypertrophied.
- FIG. 3. Thrombosis of auricle. The left auricle is completely obstructed by a thrombus loosely united to the underlying intact endocardium. The thrombus still shows distinct platelet columns but the leukocytes at their margins are disintegrating. $\times 60$.
- FIG. 4. Chronic auriculitis. The endocardium of the left auricle is irregularly thickened by a dense cellular infiltration of polymorphonuclear leukocytes, lymphocytes and large mononuclear cells. The surface endothelium is swollen. $\times 460$.



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2



3



4

Wilens and Sproul

Spontaneous Cardiovascular Disease. I

PLATE 38

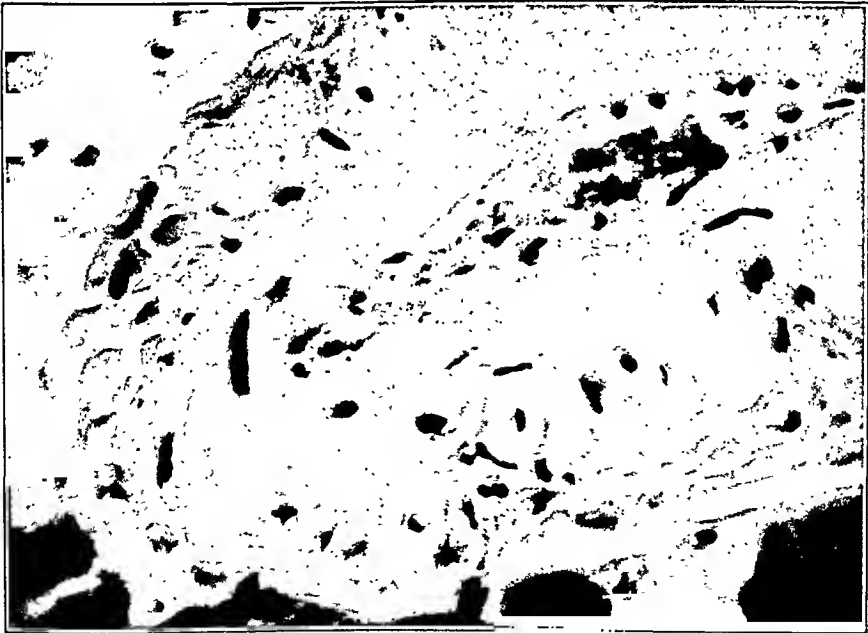
- FIG. 5. Acute bacterial endocarditis. The remains of the mitral valve leaflet are heavily infiltrated with polymorphonuclear leukocytes. The surface is ulcerated and on one aspect a vegetation containing small, deeply staining bacterial colonies is deposited. $\times 110$.
- FIG. 6. Calcification of coronary artery. The calcium is in the form of a narrow but continuous band extending around most of the circumference of the vessel. The deposit is confined to the intima and inner aspects of the media. $\times 60$.
- FIG. 7. Sclerosis of coronary artery. The lumen is reduced to an elliptical slit lined by intact endothelium. The media is converted into dense acellular collagen. A few strikingly vacuolated smooth muscle cells persist in its outer layers. The adventitia is also thickened by fibrous tissue. $\times 460$.



5



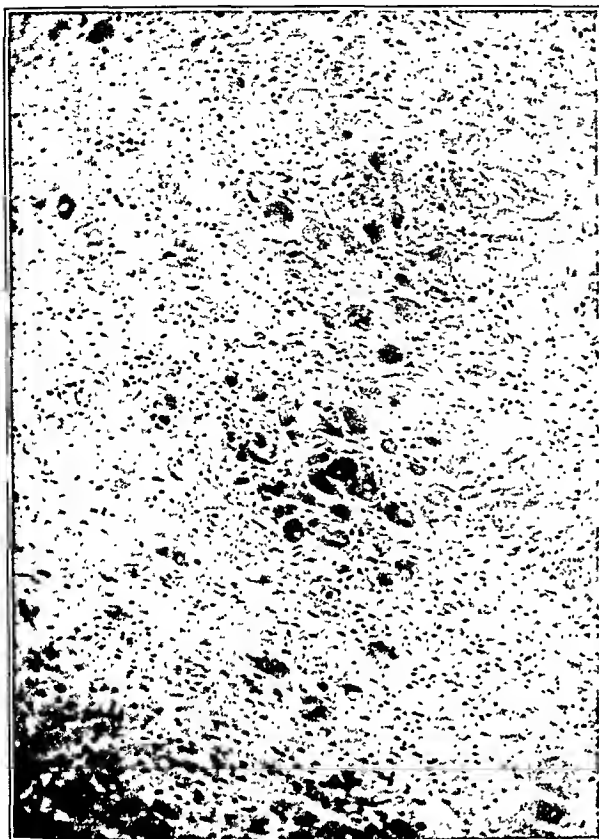
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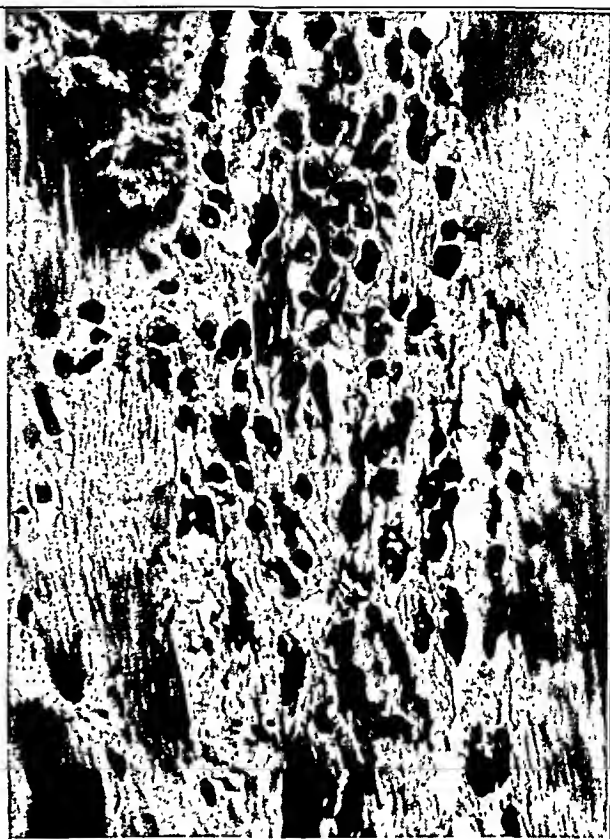
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PLATE 39

- FIG. 8. Fibrosis of myocardium. The margin of a large, dense, relatively acellular scar is shown where it adjoins and extends between atrophic and hypertrophic muscle bundles. $\times 110$.
- FIG. 9. "Interstitial myocarditis." A focal area of cellular infiltration separates adjacent muscle bundles. Lymphocytes, large mononuclear cells and fibroblasts are irregularly dispersed between collagen fibers. $\times 460$.
- FIG. 10. Chronic pericarditis. The epicardium is thickened, vascularized and densely infiltrated with a variety of leukocytes. A slight amount of fibrin is deposited at the surface. $\times 300$.
- FIG. 11. "Chronic endocarditis." The section is through an area of edematous thickening near the distal extremity of the leaflet. On the auricular aspect there are villus-like irregularities filled with pyknotic and fragmented nuclei. Beyond this point within the leaflet the collagen is swollen, granular, disorganized and deeply eosinophilic. $\times 300$.



8



9



10



11



II. LESIONS OF THE VASCULAR SYSTEM

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In a previous article¹ on this subject it was pointed out that information concerning spontaneous vascular disease in the rat is inadequate and incomplete. Duff² in a recent review lamented the fact that so little is known concerning natural vascular disease in common laboratory animals. Because of this lack, interpretation of the results of experimentally induced vascular changes is rendered uncertain. Most of the general articles on vascular disease in animals have omitted reference to the rat. Wolkoff³ included studies on the arteries of 3 rats in different age periods, the oldest of which was 2 years. She was unable to find any very definite changes in the intima or elastica other than slight splitting of the latter in the abdominal aorta of 1 animal. Hueper⁴ reported briefly on the incidental finding of changes in the pulmonary arteries associated with calcification in 12 of 75 adult rats.

Spontaneous intimal atheromatous lesions similar to those of man are apparently of limited occurrence in any other species except birds. Minimal and somewhat questionable lesions of this type have been described however in a few mammals, including monkeys and dogs (Fox⁵ and others). Löwenthal⁶ has reported impregnation by lipid of arterial walls in several mice but no definite intimal plaques. Nevertheless the absence of such lesions in the rat has never been conclusively established.

Some of the problems of senescence are best approached by the study of animals such as the rat whose natural life span is relatively brief. Alterations of the vascular system, particularly of its elastic tissue component as an indicator of old age have attained almost proverbial acceptance. However, it has always been difficult to distinguish those changes that are the inevitable result of aging from the consequences of disease processes. Lesions that are common to senile animals of many species are more apt to be the

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true expression of senescence than those that are peculiar to only a few. How much such changes depend on simple time factors and how much upon the physiological status of the animal remains unknown. In man with a maximum life span of about 90 years it takes many decades for evidences of senescence to appear. It would be interesting to know if the tempo of these changes is accelerated in animals with short life periods, such as the rat, so as to reproduce a picture similar to senility in man. Obviously if this were so, senescence would depend less upon the simple aging of tissues and more upon intrinsic, less readily explainable phenomena directly related to the natural life span of each species.

In an attempt to shed light on some of these problems a systematic study of vascular changes in a group of 487 rats kept under constant conditions over their entire natural life span was undertaken. The source and nature of this material as well as the vital statistics concerned have already been detailed.¹ Descriptions of the lesions affecting the coronary arteries were included in this earlier report on cardiac disease in the rat. In the present study the vessels of the lungs, spleen, liver, pancreas, adrenals, kidneys, pelvic organs, stomach, neck organs including thyroid and parathyroid, and brain were routinely studied in every case by means of single microscopic sections through each of the organs enumerated. Portions of the ascending aorta were included in every heart section. In addition, in many instances the entire aorta was sectioned. The blood vessels in other tissues such as the testes, intestine, mesentery, pituitary, bone and bone marrow were also frequently examined. Those of the extremities were not investigated.

AORTA

The normal rat's aorta is a delicate, thin walled tubular structure only slightly thicker and larger at its root than at the bifurcation. Microscopically the media contains a series of parallel, slightly wavy elastic fibers varying in number from 8 to 14. The individual elastic fibers are approximately as thick as those in the media of the human aorta but they are never quite so sinuous even in young animals. The elastic fibers are separated from each other by a double layer of smooth muscle cells so that they never overlap in an entangling fashion and the course of each fiber is readily

traced. At infrequent intervals short connecting elastic fibers branch off at acute angles from the main fiber to extend between the smooth muscle cells and anastomose with immediately adjacent ones. The proportion of elastic tissue to smooth muscle is much less than in the human aorta. Connective tissue is scanty and the reticulum is delicate but forms a uniform pattern as it ensheaths the coarser elastic fibers. The most internally situated elastic fiber is separated from the overlying endothelium of the intima only by delicate reticulum. The intima therefore consists of little more than the surface endothelium and its basement membrane. The adventitia is delicate and is composed of acellular loose connective tissue. Nutrient vessels never penetrate the media even at its outer aspect and indeed are not seen in the adventitia itself throughout the entire length.

With advancing age the aorta developed only relatively mild changes. It was somewhat increased in length and circumference. The adventitia was thickened and its connective tissue more compactly arranged. The media was also considerably thicker but its architecture was essentially unaltered. The intima remained as delicate as in young animals. The only lesion of note was the rare occurrence of small masses of calcium usually in the inner third, sometimes protruding into the lumen through disrupted elastic lamellae although always surmounted by intact endothelium. Such calcification was observed in 12 aortas from 9 males and 3 females. All except 1 of these were older than 700 days. The most common sites were in the lower abdominal region, near the bifurcation, at the upper limits of the sinuses of Valsalva and at the angles formed by the orifices of large branches. The calcified masses rarely exceeded 100 μ in diameter and never were more than three such deposits found in any one aorta.

One of the few invariable consequences of aging in man is alterations in the elastic tissue, chiefly a straightening and loss of waviness of the individual fibers. This is associated with enlargement of the vessels and loss of inert elasticity. It has been shown⁷ that this change is directly proportional to age and is not affected by intimal atheromas. For this reason the pattern of the elastica of young and old rats was compared to see if analogous changes occurred in the 3 year period that constitutes the life span of this species. Obviously if at the end of this period the rat has under-

gone changes comparable to senility in man one might well expect its elastic tissue to show evidences of degeneration.

Such a comparison offers difficulties for two reasons. First, the elastic fibers of young rats are not so coiled as in the young human aorta, possibly because in the former the aorta does not undergo as relatively great an excursion with each pulsation. Secondly, the relatively large proportion of smooth muscle tissue in the rat's aorta may serve to hold the elastic fibers in unnatural positions after death. The degree of contraction and postmortem rigor would thus play a more important rôle in the rat than in the human in determining the appearance of the elastic fibers. Nevertheless, examination of a large number of preparations stained by Weigert's elastic tissue method revealed mild but constant losses in waviness of the elastic fibers in old rats, as compared to those of young ones. If this change be considered an expression of senescence, then a 3 year old rat is in a physiological state comparable to that of an old man, at least as far as its aortic elastica is concerned. Moreover, the changes in these fibers are not necessarily the result of simple physico-chemical reactions occurring at fixed time intervals but are dependent upon natural life span of the species.

THE ARTERIES

In general, the arterial system of the rat developed no such constant morphological changes with increasing age as that of man. The only two exceptions were the coronary and pulmonary arteries. All other vessels, including the renal and cerebral arteries, usually retained the same characteristics in the senile rat as in the adolescent one. The intima remained thin and delicate. The elastica did not become frayed or reduplicated. The smooth muscle of the media was essentially unaltered. The vessels increased slightly in size but were never stiffened or tortuous.

The failure to form definite lipoid-containing atheromas in the intima was striking when contrasted with the usual findings in man. This did not appear to be associated with inability to deposit cholesterol in other sites. Indeed it was a common occurrence to find considerable quantities of cholesterol free, in crystalline form, and finely divided in droplets within phagocytes in areas of old inflammation. This was particularly true in the walls of old pul-

monary and uterine abscesses. Occasionally, even in otherwise normal lung tissue, there were found groups of subpleural alveoli filled with fat-laden phagocytes. The failure of atheromas to develop would therefore seem to depend less upon the inability to mobilize and deposit cholesterol than upon local factors obtaining within the arterial system. Special lipid stains were not prepared routinely so that it is impossible to exclude the possibility of subintimal impregnations by lipid, such as were described by Löwen-thal⁶ in a number of mice. Some of the mouse deposits were associated with inflammatory changes of the vessel walls.

TABLE I
Incidence, Age and Sex Distribution of Calcification of Arteries

Artery	No.	Per cent	No. of males	No. of females	Average age	
					Males	Females
Spermatic	49 *	58.4	—	—	days 745	days —
Pulmonary	224	46.0	114	110	724	768
Coronary	17	3.5	14	3	756	850
Aorta	12	2.5	9	3	803	911
Renal	8	1.6	8	0	754	—
Mesenteric	6	1.2	3	3	843	864
Cerebral	2	0.4	2	0	792	—

* The testes of only 83 males were examined microscopically.

Calcification of Arteries: Although generalized manifestations of degeneration were usually lacking, the one most common pathological alteration observed was calcification. In order of frequency calcification was noted in the spermatic, pulmonary, coronary, renal, mesenteric and cerebral arteries, as well as in the aorta itself. The calcium was usually deposited in small solid masses. The earliest portions of the vessel involved were the inner layers of the media, sometimes with impregnation of the elastic lamella alone. Larger deposits extended throughout the media and protruded through the intima into the lumen. The calcification was never very extensive and seldom did more than 3 arteries in any 1 animal have such deposits. In Table I the incidence, age and sex distribution of the findings are recorded. Males showed arterial

calcification more frequently than females. The average age for each group exceeded that of the entire series which was 702 days for males and 746 days for females.

The intratesticular branches of the spermatic artery were calcified in 49 of 83 testes that were examined microscopically. Not only was this the most frequent site but the extent of calcification was greater than elsewhere. The calcification was confined to the media which was frequently converted into a continuous ring of calcium. The deposits occurred in vessels that were otherwise normal, the intima remaining intact and delicate. They were seldom detected in animals less than 500 days old at death. The adjacent tubules did not show changes comparable to those of the senile testis in man. The basement membranes were thin, spermatogenic epithelium abundant, and interstitial cells not increased. Next in frequency of calcification were the pulmonary arteries. Here the process appears to be definitely related to sclerotic changes in the vessel wall and will therefore be described separately in detail. Identical lesions have been briefly described in 12 out of 75 adult rats by Hueper.⁴

Pulmonary Vascular System: The pulmonary arteries of the rat were peculiarly susceptible to degenerative changes. These were not unlike the ones involving the coronary arteries but were often more widespread and severe. The essential lesion consisted of atrophy of the smooth muscle coat and replacement by fibrous tissue leading to irregular thickening of the wall. In less involved areas the smooth muscle often appeared hyperplastic. The chief differences from the coronary artery lesions were that they were found in almost every rat over 2 years of age and were commonly associated with calcium deposition. The lesions developed at all points in the course of the vessels and were even continuous throughout to the smallest arteriolar radicles. Usually, however, the proximal supravalvular portions were not conspicuously involved.

The pulmonary veins were unchanged but they did exhibit an anatomical peculiarity already described in the literature by Lauche⁸ and by Takino.⁹ The outer aspect of the pulmonary veins consists of several layers of cardiac muscle bundles having all the characteristics of ordinary heart muscle. Sections through the pulmonary veins at their entrance into the heart show that this

muscle is directly continuous with that of the left auricle. Even deeply within the pulmonary tissue the smaller veins have an outer coat of cardiac muscle. This is separated internally from a quite thin and uneven layer of smooth muscle by loose connective tissue. The significance of the extra coat of aberrant cardiac muscle is not apparent but perhaps cardiac impulses are directly transmitted to the pulmonary circulation.

Direct connection between the deposition of calcium and the severity of local sclerotic changes in the artery wall could not always be established. Often calcium masses were found in vessels that were otherwise normal. More often still such changes as could be recognized in the adjacent vessel wall might well have developed subsequent to calcification. The calcium showed a striking tendency to deposit at points of bifurcation or in the angle formed by the origin of a large branch. Another prominent feature was the projection of the calcium as jagged spurs into the lumen and over which no endothelial covering was detected. In many instances these deposits completely bridged the lumen, being attached to the artery wall at opposite sides and subdividing the original lumen into two smaller ones. Yet in no instance did such obstructing plugs incite the formation of thrombi. It was not unusual to find several calcified arteries in a single microscopic section. The larger arteries at the hilus of the lung were most often involved. No relation to the frequently coexistent bronchiectatic lesions could be demonstrated. Calcified arteries were found in 24.1 per cent of the animals whose pulmonary tissue was entirely normal. Arteries traversing the walls of large old abscesses were no more frequently calcified than those remotely situated.

Calcification of the pulmonary arteries was found in 224 or 46 per cent of the rats. The incidence would undoubtedly have been still greater if a more extensive microscopic examination had been carried out. 50.7 per cent of the males were involved and only 40 per cent of the females. The lesion was seldom found in animals less than 400 days of age at death and became progressively more prevalent until the 700th day of life, when its maximum incidence was attained.

The composition of the diets on which the animals were fed varied in calcium content, depending on the relative proportions of wheat and whole milk powder in the ingredients. Sherman and

Booher¹⁰ have shown in this strain of rats that the total amount of body calcium in the growth period varied directly with the amount consumed. For this reason it was suspected that calcification of arteries might be influenced by the calcium content of the diet. When the animals were separated into two groups, one that had received diets containing about 0.19 per cent calcium, and the other with 0.33 per cent calcium, no difference in the incidence of arterial calcification between the two groups was noted. 55.4 per cent of those fed with the higher and 55.6 per cent of those with the lower calcium-containing diets had calcified pulmonary arteries. The reason why the percentage of calcification of both these groups is higher than that of the total series is that many of the animals dying at a young age were fed diets whose calcium content was not ascertained and are therefore excluded. Although the variations in calcium content cited above are not marked enough to exclude a possible dietary influence on the development of this lesion, it is obvious that in the present series its presence or absence did not depend upon the difference in calcium intake. It seems more likely that arterial calcification was a manifestation of local disturbance of calcium metabolism. The tendency to precipitate this mineral in extravascular situations as well, was quite prominent. Renal and vesical calculi were common and the bronchial cartilages were often calcified. The walls and contents of old abscesses were usually impregnated. The nature of this disordered calcium metabolism is not apparent. Although the parathyroid glands of senile rats often appeared enlarged and hyperplastic the bones showed no evidence of demineralization. The vitamin D content of the diet was not excessive.

Periarteritis: The arterial system of the rat is subject to a specific, often widespread inflammatory disease that has many of the attributes of periarteritis nodosa as it occurs in man. Lesions of this nature have been described in many different species. Nieberle¹¹ cited reports of its occurrence in cattle, swine, dogs and wild deer. Löwenthal⁶ described several instances in which single arteries of old mice showed inflammatory changes of the same type. However no record of the lesion in the rat has been found.

In the present series 47 animals or 9.7 per cent exhibited evidence of the disease. Its preponderance in the female is noteworthy. 30 were females with an average age at death of 856 days

and 17 were males averaging 700 days in age. The incidence in different age groups was as follows: under 500 days, 0 per cent; from 500 to 700 days, 3 per cent; from 700 to 900 days, 13.1 per cent; and over 900 days, 15.7 per cent. Like so many of the other cardiovascular disorders in this species, the lesion was not found until late middle life and became more prevalent as age increased.

The lesion was apparently a long standing one and various stages in its development could be recognized. In 19 animals only acute or subacute lesions were found. In 6 there were only completely arrested and healed residua. In the remainder both healed and fresh lesions were intermingled. This latter finding was an indication that the disease may progress by a series of recrudescences. The extent and distribution of the process varied considerably although there were certain sites of predilection. When only 1 or 2 vessels were involved the process was classified as localized. When more than 2 arteries in different organs showed changes it was designated as generalized. 26 fell into the former category and 21 into the latter. Such a division is only approximately accurate since recognition of the lesion depended to a certain extent upon fortuitous microscopic sections. The changes were recognized grossly when the mesenteric arteries were extensively involved or when small aneurysmal outpouchings of arteries occurred elsewhere. The mesenteric arteries often showed striking alterations. The entire mesentery was enlarged and traversed by ropy, thick tortuous vessels which often appeared entangled with one another. The individual arterial branches were greatly enlarged. In fact they often exceeded the aorta itself in diameter. All along their course they were beaded by nodular protrusions which on closer examination were revealed as a series of aneurysmal dilatations. Many of these were occluded by thrombi. The earlier, more acute lesions were detected only on microscopic study. As a rule both large and medium sized arteries were attacked, the smaller arteries less often and the arterioles almost never. The aorta itself was spared. Lesions were at one time or another identified in almost every organ and tissue which were regularly examined save only in the lungs and brain. The frequency of involvement of various arteries was as follows: unidentified mediastinal and cervical, 21; mesenteric, 15; coronary, 15; pancreatic, 14; splenic, 11; renal, 11; gastric, 4. In addition the bronchial, hepatic, adrenal, uterine,

spermatic, ovarian, peripelvic and subcutaneous arteries showed the lesion on one or two occasions.

Microscopically there was much individual variation in the appearance of the lesions. A reconstruction of the pathogenesis of the process interpreted from all available material is as follows: The earliest change was the appearance of inflammatory cells in the adventitia often in eccentrically placed crescentic masses about the larger arteries and completely circumventing smaller ones. Multiple but discrete formation of such granulomas might appear along the course of a single vessel, producing a beaded effect. The cells consisted of mixtures of lymphocytes, plasma cells, monocytes, polymorphonuclear neutrophils and usually a few eosinophils. Often their nuclei became pyknotic and fragmented so that recognition of cell types was difficult. The cells lay between connective tissue fibrils, spreading them apart. Often the inflammatory changes encroached upon the outer aspects of the media, destroying smooth muscle cells. Before it penetrated the entire width of the media the endothelial lining might be elevated by a subintimal deposit of fibrin.

Still later the changes became continuous throughout the entire vessel wall, obliterating normal structures, destroying the media completely, disrupting elastic lamellas, and causing marked irregular thickening and narrowing of the lumen. The latter was often partly or entirely thrombosed. In some areas where the fixed tissue was most severely damaged aneurysmal widenings were common and there were sometimes hemorrhagic foci. Complete rupture was not seen. Usually by the time the process reached this stage considerable connective tissue had been formed in the adventitia providing new support. Healing was accomplished by a disintegration of the infiltrating leukocytes and replacement by dense scar tissue. The thrombi became organized and sometimes recanalized. The fibrous tissue assumed a hyaline appearance and on occasion calcium deposits were superimposed. There was some regeneration of smooth muscle but only fragments of the elastica persisted. All stages of development could be found in one animal and sometimes in a single artery. Areas of healing were not immune to secondary flareups and often an early fresh lesion was added to an older organizing one.

Save only in the case of the kidney, secondary manifestations

in the viscera due to obstructed blood supply were surprisingly infrequent. The gut was sometimes mottled by hemorrhagic areas. Focal necroses and interstitial scars appeared in the spleen, pancreas and myocardium. Definite infarction was not encountered. In the kidneys, however, there were often widespread tubular and glomerular changes indistinguishable in many respects from true glomerulonephritis. The convoluted tubules were widely dilated, lined by flattened epithelium, and obstructed by hyaline casts. Other areas of interstitial fibrosis and tubular atrophy although usually less frequent did distort the normal architecture. The glomeruli were in various stages of fibrosis and irregularly distributed in the cortex. Some were enlarged, others shrunken and completely hyalinized. Adhesions of tufts to the capsules of Bowman were numerous. The basement membranes of the capillary loops were thickened and the tufts themselves ischemic. Epithelial proliferation and cellular infiltration were never pronounced. Such changes in the kidney closely simulating if not identical with true glomerulonephritis were found in 9 of the 47 cases. In 13 others there were isolated areas of renal atrophy and fibrosis not unlike the scars resulting from arteriosclerosis of large renal arteries in man.

The etiology of this disease was not determined. Suppurative lesions were extremely common. In 18 of the 30 females there were large uterine abscesses. Similar foci of suppuration occurred with equal frequency in the absence of inflammatory arterial disease. Except for the involvement of the coronary arteries there was no association with endocardial or other intracardiac lesions. A possible relation to diet is disclosed by the fact that the disease appeared in only 1 animal out of 75 receiving meat and vegetables. In this animal the process was localized in the mesenteric artery. Among 356 rats known to lack either of these ingredients in their diet, there were 46 cases. The diet of these animals was less varied and consisted largely or entirely of dried milk powder and ground wheat. This discrepancy cannot be attributed to differences in longevity as 48 of the 75 animals in the first group survived longer than 700 days. Neither can it be ascribed to differences in susceptibility to suppurative infections since these occurred with approximately equal frequency and severity in both. It is thus suggested, although by no means proved, that dietary differences

may influence the incidence of this disease. It would require more extensive observations on a larger series of rats with the diets regulated from this point of view to establish the proposition.

There are many circumstances which make it seem probable that this disease is closely related to periarteritis nodosa in man. Certainly the histological features, the distribution of the lesions, the mode of onset and development, and the permanent residual deformities are closely analogous to those of the human disease. The pulmonary and cerebral arteries in both species are seldom involved. The mesenteric, renal and coronary arteries in both are often damaged. Again the vulnerability of medium sized arteries is a finding common to both. Renal lesions closely resembling glomerulonephritis have been described in association with the human form of periarteritis nodosa.¹² The occurrence of similar vascular lesions in many other species may indicate that they are all etiologically related. The only striking dissimilarities of rat to human periarteritis are in age distribution and incidence. The human disease occurs at all periods of life, whereas the rat lesion is pretty well limited to old animals. Human periarteritis is comparatively rare but in the rat it is one of the most common forms of systemic vascular disease. Neither of these discrepancies offers insurmountable evidence against the identity of the two processes.

Renal Lesions: The kidneys of senile rats are seldom entirely normal. In addition to the 9 cases of nephritis associated with periarteritis, there were 10 others in which the kidney cortex showed evidence of widespread degeneration involving both tubules and glomeruli. The only essential difference from human glomerulonephritis was the paucity of either proliferative or exudative reactions in the glomeruli and the fact that the kidneys were not contracted. Fine granulations of the cortical surface were sometimes visible with a hand lens. Another finding of high incidence was pelvic calculi leading to hydronephrotic atrophy. The kidneys were also subject to bacterial infection in many cases. Some of the older areas of scarring and atrophy were undoubtedly due to healed pyelonephritis.

Because of these complicating factors it was difficult to establish the extent to which the degenerative lesions encountered were dependent upon vascular disease. The major renal arteries seldom

deviated from normal although calcification was noted 8 times. The intima in senile rats was not thickened and the elastic fibers did not split or become multiple as is so often the case in adult man. Nevertheless there were many instances of linear or wedge shaped scars in the parenchyma directed at right angles to and retracted below the cortical surface. These had all the attributes of arteriosclerotic scars in the human kidney. In addition, a few scattered glomeruli were often hyalinized. Lesions of this nature were found in 65 males and 51 females, a total incidence of 23.8 per cent. They were uncommon in animals younger than 700 days at death and present in 34.6 per cent of those surviving more than 900 days. Because of the absence of sclerotic changes in the renal arteries it is impossible on morphological grounds to associate the scarring with impaired circulation. Some must have resulted from healed pyelonephritis, others possibly from pressure on the arteries in the peripelvic tissue exerted by intrapelvic calculi. This latter suggestion is not entirely implausible inasmuch as the peripelvic tissue in the rat is lacking in adipose tissue which might cushion and protect the vessels. The arterioles in and about the scars often seemed to have thick muscular walls and only minute lumens, but hyaline necrosis was not observed.

THE ARTERIOLES

In general, the arterioles showed little pathological change. In no instance were arteriolar sclerotic lesions disseminated throughout the splanchic area, as is seen so frequently in association with human hypertension. There is no reason for believing that any of the rats had had elevated blood pressures comparable to a primary hypertension. Hypertrophy of the heart occurred only in conjunction with other cardiac lesions or in animals that had severe renal lesions. As previously noted, in scarred kidneys the arterioles sometimes appeared thickened and tortuous. Definite hyalinization of the renal arterioles was observed in 3 cases and in only 1 of these were the changes striking. In this particular animal, a 1005 day old female, there were associated widespread glomerular changes which might have been secondary to the arteriolar lesion. The renal changes were suggestive of arteriolar nephrosclerosis but extrarenal arteriolar lesions were not observed.

The splenic arterioles were examined closely inasmuch as these

are so constantly altered in man. In only 1 animal, an 800 day old female, were comparable lesions found. In the spleen of this particular animal practically every follicular arteriole was thickened and contained a subintimal accumulation of deeply eosinophilic, homogeneous material. In the spleen of 1 other rat, an 850 day old male, a few central arterioles showed similar changes. In all the other old animals the splenic arterioles were perhaps thicker and more tortuous than normal but there were no definite sclerotic changes.

The pulmonary and bronchial arterioles often exhibited striking muscular hypertrophy. This, however, was usually associated with more profound changes in the larger arteries.

THE VEINS

The only finding of note in the veins was the occasional thrombosis of the hepatic, adrenal, splenic, renal, uterine and pulmonary vessels. The thrombi were at times secondary to lesions in the adjacent tissue but also formed in normal tissue without obvious cause. Small pulmonary emboli were present in a few cases and were probably derived from such thrombi. With advancing age none of the veins examined showed degenerative changes of any consequence.

SUMMARY AND CONCLUSIONS

The pathological manifestations of vascular disease in 487 rats of all ages in which death occurred as the result of natural causes are described. Intimal lesions of the arteries comparable to those of man and birds, or those experimentally induced in rabbits following cholesterol ingestion, or in the coronary arteries of rats with administration of excessive doses of vitamin D (Ham and Lewis¹³), were not observed. The elastic fibers in the aortas of senile rats were thicker and less undulating than those of young ones. Except for the absence of fraying and splitting, this change is analogous to that in the elastic fibers of man with advancing age. If it be interpreted as an indication of reduced elasticity, the assumption can be made that degeneration of elastic tissue is dependent upon the natural life span and not upon simple aging of this tissue. Since intimal thickening did not accompany this medial change, as it so commonly does in man, it seems likely that the two

are unrelated and that the latter is not a true phenomenon of senility.

Only the coronary and pulmonary arteries were commonly the seat of degenerative changes. These consisted of fibrosis of the media and thickening of the wall. In the pulmonary arteries the lesion was frequently associated with calcification. Calcification was found also in other arteries, particularly the spermatic.

A specific inflammatory disease of arteries identical with or at least closely resembling periarteritis nodosa in man was found in 9.7 per cent of the animals.

Renal lesions similar to arteriosclerotic atrophy in the human kidney were described but their association with vascular disease could not be established. The arterioles showed evidences of sclerotic changes in only a few exceptional cases and in none was generalized arteriolarsclerosis recognized.

All of the lesions encountered were influenced by age, few of them being observed before the 700th day of life. All of the non-inflammatory lesions were more common in males.

The absence of amyloidosis in all of the animals is particularly noteworthy in view of the success with which this change has been experimentally produced in rodents by a variety of methods.¹⁴ The circumstances would seem to have been especially propitious for its development spontaneously in these animals. Chronic suppurative lesions were very common and one of these, infection of the auditory bullae, was often associated with osteomyelitis of the adjacent bony structures sometimes with extension into the cranial cavity.

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DESCRIPTION OF PLATES

PLATE 40

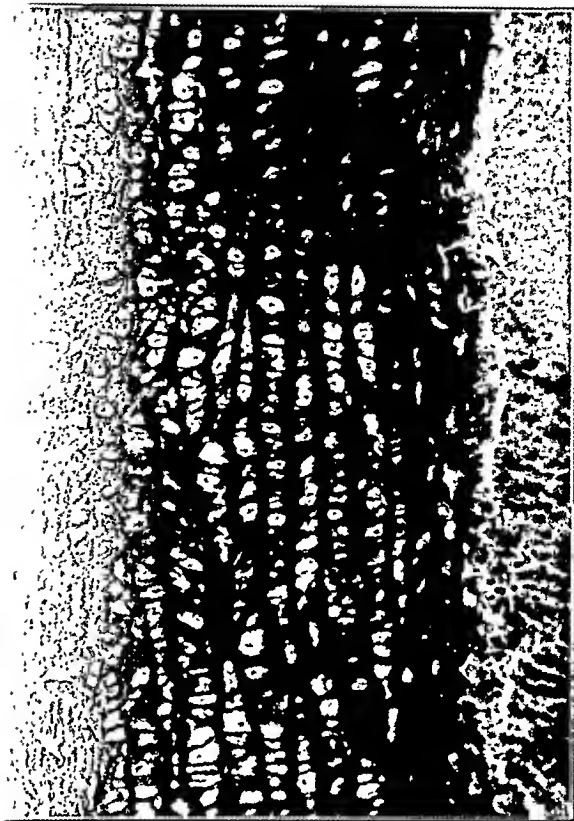
FIGS. 1 and 2. Elastica in media of aorta. The photographs are from comparable points in the ascending aorta. Fig. 1 is from a 93 day old and Fig. 2 from a 1021 day old rat. In the senile animal the media is much broader, the individual lamellae slightly thickened and much farther apart. The most striking change, however, is the stretching and loss of undulation of the elastic fibers in Fig. 2, as compared to those in Fig. 1. Weigert's elastic tissue stain. $\times 300$.

FIG. 3. Calcification of pulmonary artery. Two solid masses of calcium are embedded in the intimal surface of the sclerotic vessel. The wall of the artery at this point is irregularly thickened by dense fibrous tissue which has replaced the smooth muscle. $\times 300$.

FIG. 4. Calcification of abdominal aorta. At the orifice of a large arterial branch a solid mass of calcium is deposited and protrudes into the lumen. $\times 100$.



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PLATE 41

FIG. 5. Calcification of spermatic artery. Calcium is deposited as curved plates in the media of the vessel without causing the latter to become thickened. $\times 110$.

FIG. 6. Acute periarteritis of small artery. The entire adventitia of the vessel is heavily infiltrated by lymphocytes, polymorphonuclear leukocytes, large mononuclear cells and pyknotic nuclei. The media is degenerating and a subintimal deposit of fibrin has been precipitated. The lumen is still patent. $\times 460$.

FIG. 7. Acute periarteritis of large peripancreatic artery. An acute inflammatory reaction attended by the fragmentation of nuclei of infiltrating cells is apparent in the adventitia and outer aspects of the media. The nodular character of the lesion is self evident. $\times 110$.

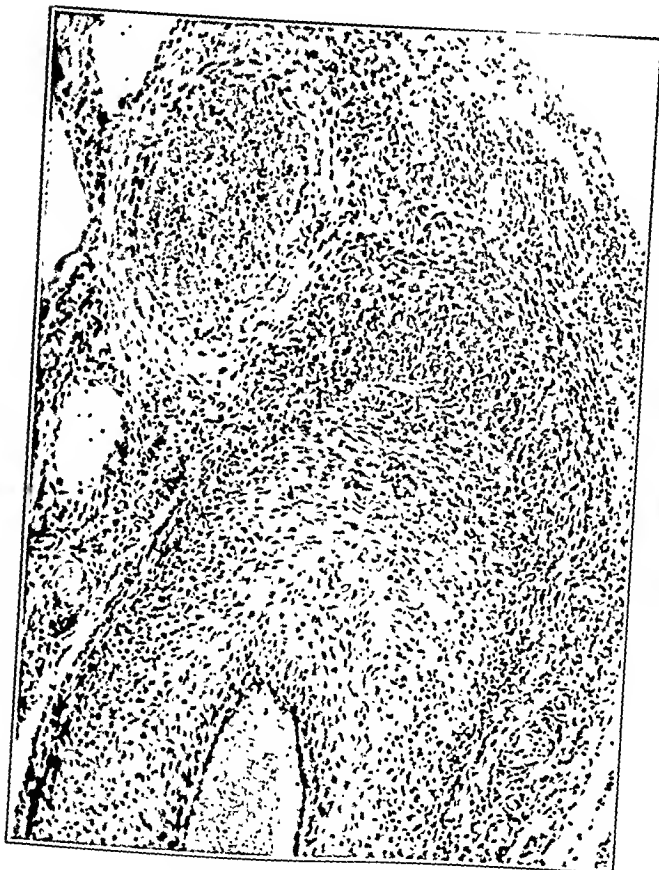
FIG. 8. Chronic periarteritis of mesenteric arteries. The arteries throughout the mesentery to their points of entrance into the intestinal wall are greatly enlarged, twisted, tortuous and nodular. Aneurysmal dilatations are numerous.



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PLATE 41

FIG. 5. Calcification of spermatic artery. Calcium is deposited as curved plates in the media of the vessel without causing the latter to become thickened. $\times 110$.

FIG. 6. Acute periarteritis of small artery. The entire adventitia of the vessel is heavily infiltrated by lymphocytes, polymorphonuclear leukocytes, large mononuclear cells and pyknotic nuclei. The media is degenerating and a subintimal deposit of fibrin has been precipitated. The lumen is still patent. $\times 460$.

FIG. 7. Acute periarteritis of large peripancreatic artery. An acute inflammatory reaction attended by the fragmentation of nuclei of infiltrating cells is apparent in the adventitia and outer aspects of the media. The nodular character of the lesion is self evident. $\times 110$.

FIG. 8. Chronic periarteritis of mesenteric arteries. The arteries throughout the mesentery to their points of entrance into the intestinal wall are greatly enlarged, twisted, tortuous and nodular. Aneurysmal dilatations are numerous.



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PLATE 42

FIG. 9. Nephritis in periarteritis nodosa. The architecture of the cortex is disarranged. Some of the tubules are enlarged and obstructed by hyaline casts. Others are atrophic and have shrunk into the increased interstitial connective tissue. The latter is infiltrated by lymphocytes. The glomeruli are distorted and swollen so that the tufts obliterate the capsular spaces. The tufts are ischemic, compact and depleted of cells. $\times 110$.

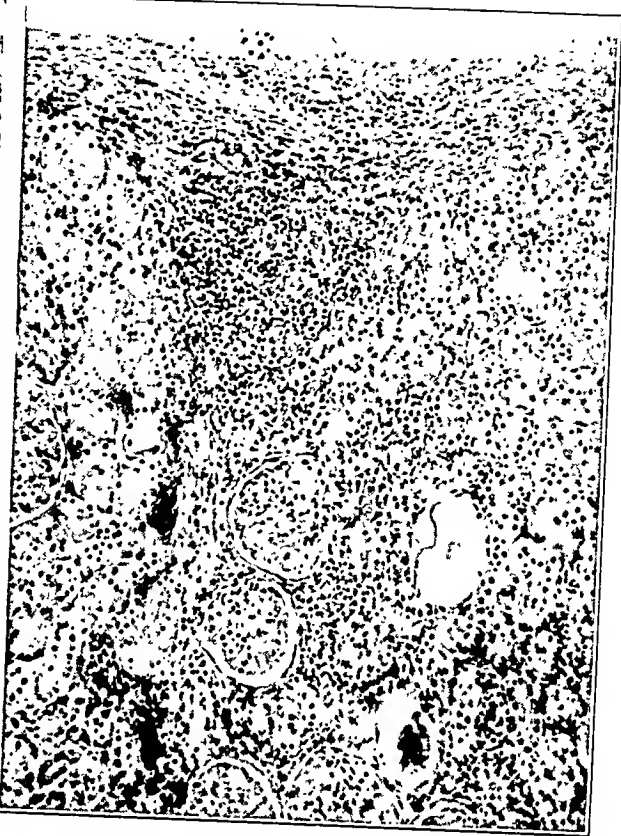
FIG. 10. Atrophy and fibrosis of renal cortex. The edge of a wedge shaped scar borders on adjacent intact renal cortex and merges with the slightly thickened and sunken capsule. The tubules in the scar are completely atrophic and the glomeruli are shrunk and partly replaced by fibrous tissue. A heavy lymphocytic reaction has occurred. $\times 110$.

FIG. 11. Hyalinization of splenic arterioles. The lumens are greatly narrowed. The walls are thickened by dense homogeneous masses of hyaline material lying between the endothelium and the outer layers of the vessel wall. $\times 110$.

FIG. 12. Marked smooth muscle hypertrophy in the media of a pulmonary arteriole. The lumen is greatly reduced. A small capillary is seen as it emerges directly from the arteriole. $\times 300$.



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Wilens and Sproul

Spontaneous Cardiovascular Disease. II

TRANSMISSION OF CHLOROLEUKEMIA OF MICE *

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Since Burns described the first case of chloroleukemia in man in 1825, 175 cases of this disease have been reported up to 1937.¹ Early reports considered chloroleukemia to be of the lymphoid type, but recent cases have all been classified as myeloid in type.^{2, 3}

Chloroleukemia differs from other leukemias only in the green color of the leukemic nodules and lymph nodes. The shade of green varies in different cases and even in the different tissues of the same case. The green color has been attributed to the presence of lipochromes,⁴ porphyrines,⁵ and the eosinophils,⁶ which abound in some cases of chloroleukemia. Treadgold⁷ believes that the green color is possibly due to a degeneration of the granules of the myelocytes and myeloblasts, aided by the products of hemoglobin disintegration. It has been suggested by Kossel and Giese⁸ that the green color depends on the presence of both free sulphur ions and a certain amount of iron in a reactive state.

Chloroleukemia has been reported in several of the domestic and laboratory animals, including the pig, common fowl, rat and mouse. The term chloroleukemia in fowl, as used by Mathews,⁹ is a misnomer, since the multiple tumors of the fowl diagnosed by him as chloroleukemia have a white color and are not known to assume a greenish hue.

Wilens and Sproul¹⁰ reported 12 cases of spontaneous leukemia in the rat, 1 of lymphoid and 11 of the myeloid type. In 4 of the myeloid cases the leukemic tissues were light green in color.

Simonds,¹¹ describing the 67 cases of leukemia in the first 15,000 autopsies of the Maud Slye strain of mice, found no cases of chloroleukemia. The only case of this disease in mice was reported by Hill,¹² in an article describing lymphoid hyperplasia in 215 mice. She describes one mouse with "chloro-myelo-sarcoma," in which there was a mixed myeloid and lymphoid invasion of the

* This investigation has been supported by grants from the Lady Tata Memorial Trust and the International Cancer Research Foundation, and by a fund for the study of leukemia.

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lymph nodes and spleen. The blood picture, however, was myeloid in nature. No attempt was made to transmit the disease.

The transfer of chloroleukemia and the anatomical characteristics of this disease in mice are the subject of this paper.

A case of chloroleukemia in a mouse was observed in this laboratory in November, 1936. Hundreds of cases of spontaneous lymphoid leukemia, and numerous cases of myeloid leukemia, have occurred here, but the green color of the leukemic infiltrations has been observed only in very few mice. This mouse, Slb 351, was a female, born Nov. 3, 1935, and dying Nov. 8, 1936. At postmortem the spleen was grayish red and enlarged to 3.5 by 1.2 cm. The superficial lymph nodes were enlarged to 4 to 7 mm. in the greatest diameter and were a light green in color. The internal lymph nodes were similar to the superficial lymph nodes, both in color and in size, but the mediastinal nodes were not involved. The liver was slightly enlarged and gray. In the lungs there were numerous small red areas of hemorrhage. A blood smear showed numerous erythroblasts and the white cells were estimated at 400,000 with myeloid cells in all stages of maturity, myeloblasts being especially numerous.

MATERIAL AND METHODS

The spleen and lymph nodes were removed aseptically, minced in the presence of Tyrode solution, and drawn into a syringe through cotton to filter out the larger particles. Intravenous injections of 0.1 cc. of this suspension were made into the tail vein. Subcutaneous injections of 0.2 cc. of unfiltered fragments of leukemic tissue were made into the subcutaneous tissue of the right side. Later, with the development of subcutaneous tumors, tumor material was prepared as described above and injected intravenously and subcutaneously.

Transfers were also made of splenic material which had been frozen slowly to -70° C. during one-half hour and kept at that temperature for periods ranging from 6 to 93 days. (Concerning the preservation of leukemic cells in the frozen state, see Breedis, Barnes, and Furth.¹³) The material was thawed slowly by keeping the sealed tubes containing the frozen splenic material first at refrigerator and subsequently at room temperature. A cell suspension was made of the splenic material and injected intravenously.

Differential cell counts were made on touch impressions of the leukemic organs and tumors and on blood smears stained with Wright's and Giemsa's solutions and with the benzdine oxydase stain.

EXPERIMENTAL

Approximately 2 hours after the death of mouse Slb 351, during which time it was kept in the refrigerator, the spleen and lymph nodes were separately cut up in Tyrode solution. Of 5 mice injected intravenously with splenic suspension, 3 developed chloroleukemia; while 2 mice injected intravenously with a cell suspension of lymph node developed chloroleukemia.

TABLE I

Susceptibility of Related Mice to Various Routes of Injections of Spleen, Lymph Nodes and Leukemic Tumor Tissue

Route of injection	Number of mice injected	Successful inoculations
Intravenous	82	78
Subcutaneous	13	3
Intravenous and subcutaneous	10	8

During a period of 12 months numerous transfers were made. Table I summarizes the results obtained by the injection of spleen alone, spleen and tumor, and spleen and lymph node by intravenous, subcutaneous and combined subcutaneous and intravenous injections. Of the different routes, intravenous was the best, being successful in 95.1 per cent of the injections. Subcutaneous tumors were observed following subcutaneous inoculation in 23 per cent of the mice inoculated.

The length of life following intravenous injection of splenic material averaged 21.5 days in a series of 31 mice. The longest duration of life following injection was 48 days, and the shortest 9 days. A tendency towards a decrease of length of life following repeated passages was observed. For the first 6 passages the average length of life was 26.7 days, whereas in the last 6 passages the duration of life was 16.6 days.

Subcutaneous tumors could be as readily produced by the use of

tumor tissue alone as with spleen or lymph nodes. The production of generalized chloroleukemia was approximately one-third as frequent in the mice injected intravenously with tumor cell suspension (30 per cent) as in those mice injected intravenously with cell suspensions of other tissue, spleen and lymph nodes (95.1 per cent) (Table II).

Of five mice that were given a single dose of X-ray (400 r), all developed subcutaneous tumors 14 days following the subcutaneous inoculation of a suspension of splenic tissue (Table II). While there was a rapid development of subcutaneous tumors at the site of inoculation, these mice did not show evidence of generalized leukemia until from 55 to 75 days after subcutaneous inoculation.

TABLE II

Results Obtained by the Injection of Tumor Material into Related Mice

Route of injection	Number of mice injected	Successful inoculations
Intravenous	10	3
Subcutaneous	23	5
Subcutaneous *	5	5

* Mice given 400 r preceding the inoculation.

Twenty mice of unrelated stock were injected both subcutaneously and intravenously with a cell suspension of splenic tissue. Half of these mice were irradiated (400 r). None of the mice developed leukemia. Eight mice were then injected intravenously with a cell suspension of spleen after exposure of the mice to 400 r of X-ray and 1 week later these mice were given 300 r of X-ray. At the end of 10 weeks none of these mice had developed leukemia.

Reinjections were made into mice that had failed to develop leukemia after they had been injected intravenously or subcutaneously with leukemic cells. The reinjections were made intravenously, but none of the 14 mice developed leukemia. These mice either had a natural resistance to leukemia or had been immunized by the original inoculation.

Cell suspensions were made of the splenic material which had been frozen at -70° C. and kept at that temperature for varying periods of time. Leukemia developed in 1 of 4 mice injected with

splenic material kept at -70° C. for 6 days. Of 4 mice injected with splenic material kept at -70° C. for 13 days, 1 developed leukemia; in 5 mice injected with the same material frozen at -70° C. for 94 days, leukemia failed to develop.

Of all strains of leukemia, this was found to be the most susceptible to freezing; nevertheless, a sufficient number of cells survived to produce leukemia in 2 of 13 mice injected. Further experiments on the susceptibility of these cells to freezing are in progress.

GROSS AND MICROSCOPIC EXAMINATION OF TISSUES OF MICE WITH GENERALIZED CHLOROLEUKEMIA AND SUBCUTANEOUS TUMORS

The mouse that developed the spontaneous chloroleukemia and the intravenously injected animals developed the same type of diffuse, generalized lesions, but no tumor formation was observed. The following description is characteristic of most of the mice that developed the generalized disease.

The spleen is greatly enlarged, measuring up to 3 by 1.3 by 0.8 cm., and is grayish red in color. There is generalized enlargement of the lymph nodes. The cervical, axillary, inguinal, mediastinal and periaortic nodes measure from 3 to 8 mm. in the greatest diameter and are of various shades of green. The mesenteric nodes measure 2 by 0.6 by 0.3 cm. and are similar in color to the other lymph nodes. The bone marrow is grayish red. The liver is moderately enlarged. It is light reddish brown mottled with small irregular areas of red and grayish yellow. The lungs, both externally and on the cut surface, show small irregular areas of red. In some instances the kidneys are pale red and contain minute areas of gray. The heart, adrenals, intestine and ovaries appear normal in gross.

Microscopically, the splenic pulp is extensively infiltrated by leukemic cells. These cells for the most part are of two types: (1) large cells with large, irregular, rounded, either hyperchromatic or vesicular nuclei, and very little cytoplasm; the vesicular nuclei show large acidophilic nucleoli; and (2) large cells with bean shaped nuclei in which there is a greater amount of cytoplasm than in the above mentioned cells. Mitotic figures are numerous. Very few immature leukocytes are present. The lymph follicles

are atrophic or have been replaced by leukemic cells. A moderate number of megakaryocytes are present. In the other organs and structures in which there are leukemic infiltrations, the same types of cells are present, with the exception that megakaryocytes are not present. The normal structure of the lymph node is replaced by an infiltration of leukemic cells. Small areas of beginning necrosis are present. The capsule and pericapsular tissue are also infiltrated with leukemic cells. In the bone marrow there is replacement of the normal structure by masses of myelocytes and myeloblasts. The periosteum is raised from the cortex of the bone by a thin layer of leukemic cells. This layer is in direct continuity with the leukemic cells in the marrow cavity by way of growths of leukemic cells in the central canals of the Haversian systems (Fig. 7). In the liver there is extensive infiltration of the portal spaces by leukemic cells. Small collections of leukemic cells are also present in the liver lobules away from the portal spaces. A moderate number of mitotic figures are seen in these groups of cells. There are many leukemic cells in the liver sinusoids, which contain more leukocytes than erythrocytes (Fig. 4). A few erythroblasts are also present in the sinusoids. In the kidneys there are extensive infiltrations of leukemic cells around the blood vessels in both the cortex and the medulla. The glomerular capillaries are filled with leukemic cells. The alveolar walls of the lungs are greatly thickened by infiltrations of leukemic cells, to such an extent that in some areas the alveoli are collapsed and solid fields of leukemic cells are present. Conspicuous infiltrations are seen at the hilum of the lung around the vessels and bronchi (Fig. 3).

The subcutaneous leukemic tumors present at the site of injection vary in size from 0.6 by 0.4 by 0.4 cm. to 1 by 1 by 0.8 cm. The tumors range from a grayish yellow to a light green in color, and are attached to the subcutaneous tissue and in some cases to the overlying skin.

Microscopically the leukemic cells have infiltrated the subcutaneous tissue and skeletal muscle. Many of the cells are large with very little basophilic cytoplasm. The nuclei of some are hyperchromatic, while most are vesicular with acidophilic nucleoli. There are also a few cells with bean shaped nuclei and with a large amount of cytoplasm. A very few immature leukocytes are present.

Eosinophilic leukocytes are not present in the tumor but are seen occasionally in the spleen. Mitotic figures are extremely numerous (Figs. 5 and 6).

Differential counts made with Wright's and Giemsa's stains and with oxydase stained blood smears indicate that approximately 50 per cent of the cells are myeloblasts, promyelocytes, myelocytes and metamyelocytes. There are a moderate number of annular forms and immature polymorphonuclear leukocytes present, but mature polymorphonuclear leukocytes are never more than 5 per cent (Figs. 1 and 2). In the bone marrow and leukemic tissues, studied by means of Wright's and Giemsa's methods, and oxydase stained touch impressions, 75 to 85 per cent of the cells are myeloblasts, promyelocytes, myelocytes and metamyelocytes. The subcutaneous tumors are composed of from 80 to 95 per cent myeloblasts and promyelocytes with no evidence of maturation towards myelocytes and metamyelocytes.

White blood counts done on 5 leukemic mice ranged between 136,900 and 672,000 per cmm.

OBSERVATIONS ON GREEN COLOR OF LYMPH NODES

The green color of the lymph nodes fades rapidly following exposure to air, and has entirely disappeared in 15 minutes to a half hour. When the material is preserved in Kaiserling's solution the color fades, as when exposed to the air.

An attempt was made to preserve the color, using a reducing solution of sodium hydrosulphite,¹⁴ without success. Hydrogen peroxide has often been recommended for the preservation of the green color of chloroleukemia.¹⁵ Several attempts were made to preserve the green color by the use of 3 per cent hydrogen peroxide but the green color faded more rapidly in the solution than in air.

Microspectroscopic examination of the green lymph nodes did not reveal any characteristic band.

Microscopic examination of the green colored tissues indicated that the green color was not due to the presence of eosinophils, as these cells were not present at all, or only in very small numbers.

Placing the green tissue in Tyrode solution in an atmosphere of carbon dioxide preserved the color very satisfactorily for from 3 to 4 hours.

SUMMARY AND CONCLUSIONS

A strain of chloroleukemia of mice is described that is readily transmitted to related mice by the intravenous injection of a suspension of leukemic cells.

Subcutaneous inoculation of leukemic leukocytes produces a localized tumor at the site of inoculation in approximately 23 per cent of the inoculated mice. These tumors grow very slowly. Intravenous injection of a suspension of leukemic cells produces a rapidly progressing generalized leukemia, fatal after approximately 20 days, in 95.1 per cent of the injected mice. This observation indicates that large numbers of leukemic cells are destroyed in the subcutaneous tissues of mice that are susceptible to intravenous administration of similar cells.

Suspensions of tumor cells injected intravenously are much less effective in transmitting the disease than spleen and lymph node.

Tumor tissue and splenic tissue subcutaneously injected are about equally effective in producing subcutaneous tumor nodules.

Exposure of mice to 400 r of X-ray preceding the injection results in a greater percentage of successful subcutaneous inoculations.

Unrelated mice of two different stocks are resistant to transmission of the disease. Exposure of these mice to 400 r of X-ray has not rendered them susceptible to the disease.

Mice that have been negative following intravenous or subcutaneous injection of leukemic cells have also been negative following intravenous reinjection with leukemic splenic material.

The almost complete absence of eosinophils in the leukemic infiltrations indicates that these cells are not responsible for the green color. The most intense green color is shown by the lymph nodes, while the subcutaneous tumors, which are composed almost exclusively of malignant leukemic cells, are gray with only a faint greenish hue.

NOTE: We wish to express our thanks to Dr. Jacob Furth for his many helpful suggestions and advice which aided us in the completion of this study.

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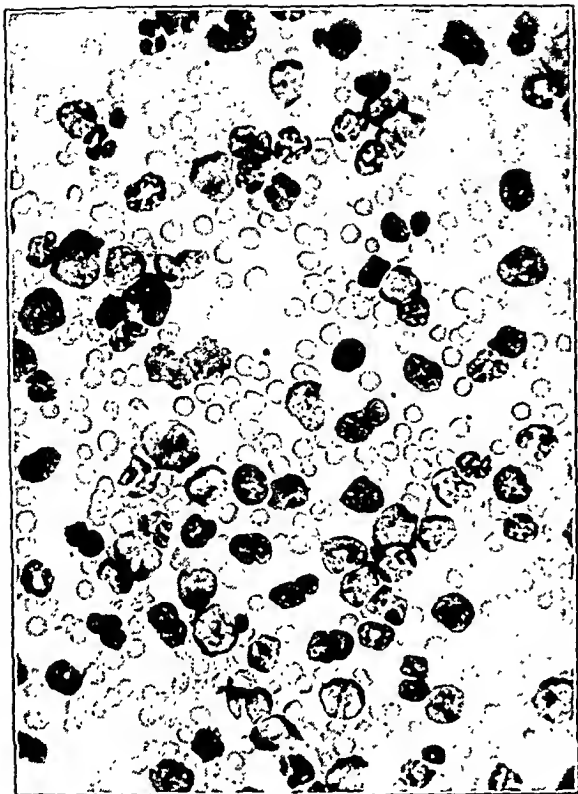
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DESCRIPTION OF PLATES

All blood smears were stained with Wright's and Giemsa's solutions and the sections with hematoxylin and eosin solutions. The magnifications stated are approximate.

PLATE 43

- FIG. 1. Blood smear from a mouse with transmitted chloroleukemia, showing myeloblasts, promyelocytes, myelocytes, premyelocytes and immature polymorphonuclear leukocytes. $\times 300$.
- FIG. 2. Higher magnification of a blood smear from a mouse with transmitted chloroleukemia, showing immature myeloid cells. $\times 900$.
- FIG. 3. Extensive myeloid infiltration of lung around a bronchiole and a blood vessel in a mouse with transmitted chloroleukemia. $\times 100$.
- FIG. 4. The liver from a mouse with transmitted chloroleukemia, showing distention of the sinusoids by leukemic cells, with compression of the liver cells and extensive infiltrations in the portal spaces. $\times 200$.



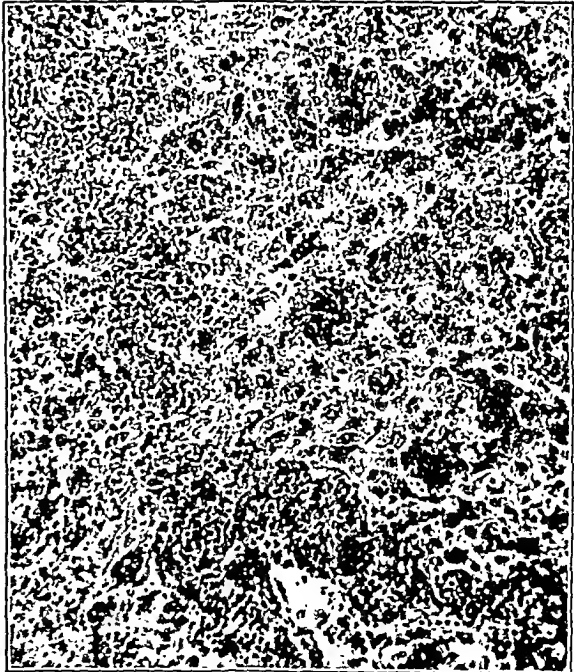
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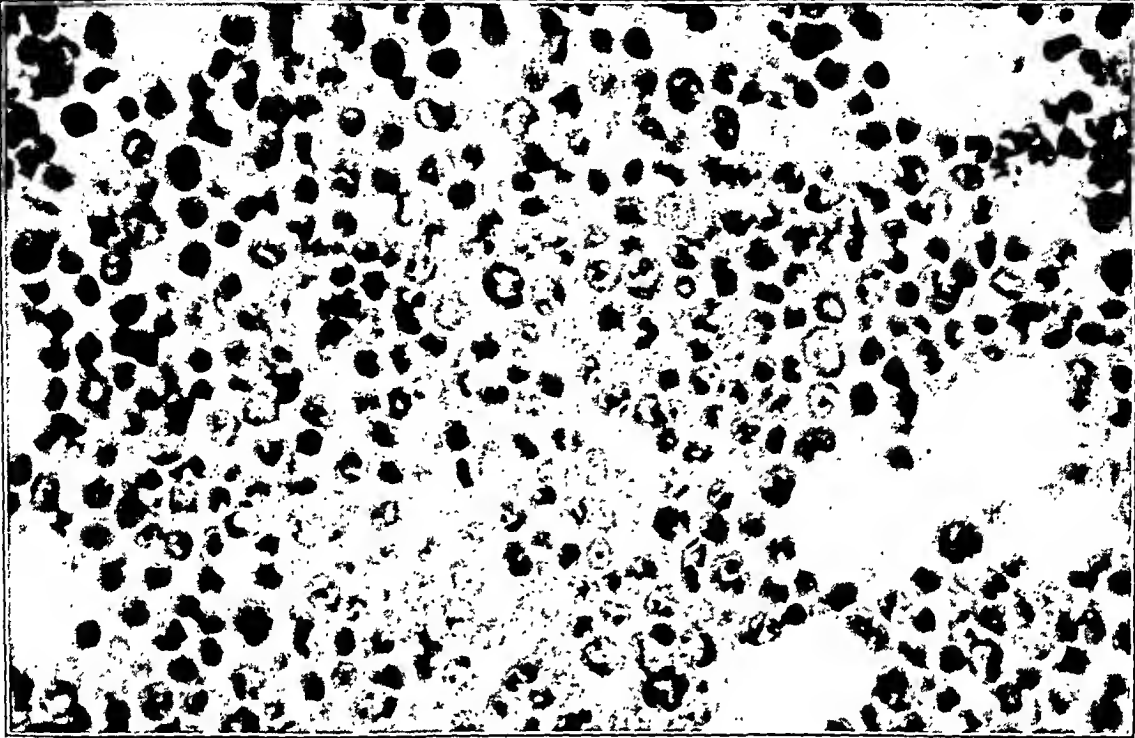
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Hall and Knocke

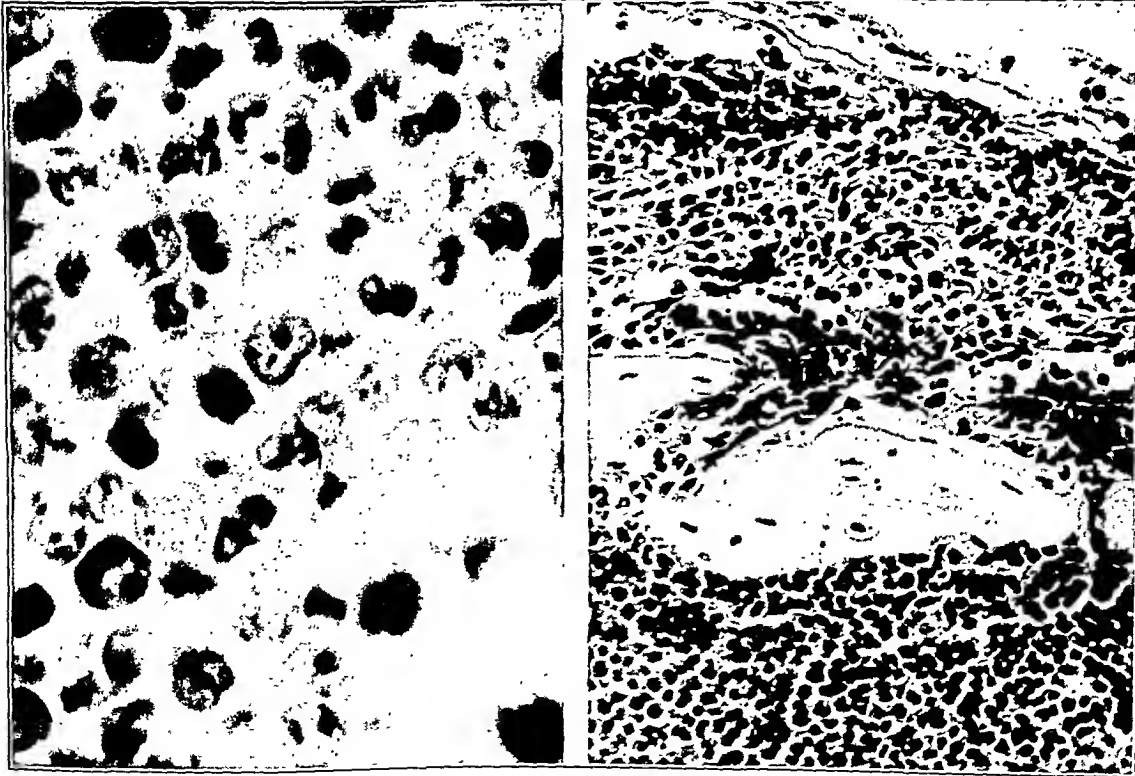
Transmission of Chloroleukemia of Mice

PLATE 44

- FIG. 5. Immature myeloid cells infiltrating the subcutaneous tissue following subcutaneous inoculation. $\times 700$.
- FIG. 6. Higher magnification of subcutaneous tumor showing myeloblasts, promyelocytes and several more mature myeloid cells. Several mitotic figures are present. $\times 900$.
- FIG. 7. Advanced infiltration of the bone marrow by leukemic cells with extension through the Haversian canals and separation of the periosteum from the cortex. $\times 200$.



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Hall and Knocke



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Transmission of Chloroleukemia of Mice

THE DISTRIBUTION OF MATERIAL FOLLOWING INTRACEREBRAL
INOCULATION INTO MACACUS RHESUS MONKEYS AND ITS
POSSIBLE INFLUENCE UPON THE RESULTS OF
NEUTRALIZATION TESTS IN EXPERI-
MENTAL POLIOMYELITIS *

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Intracerebral inoculations of monkeys with poliomyelitis virus have been employed extensively since Flexner and Lewis¹ in 1909 demonstrated that this route of inoculation is an effective method of transmitting the disease to these animals. Because monkeys have been most consistently infected with virus in this manner, the intracerebral inoculation has been considered the most reliable means of determining infectivity of the virus, especially after it has been subjected to treatment with an inhibitory reagent such as immune serum. However, despite the widespread use of injections into the monkey brain, there is little knowledge concerning the fate of the inoculum subsequent to its deposition into the brain substance.

Many investigators²⁻⁵ have studied the diffusion of material throughout the central nervous system by the injection of dyes or of India ink; but as far as we could ascertain, no experiments have been conducted with a view toward determining to what degree and extent material introduced into an area of the brain is eventually distributed, and what bearing the resultant distribution may have upon the ultimate infectivity of infectious material thus deposited.

Hurst,⁶ in a study on the pathogenesis of experimental poliomyelitis, as a preliminary step injected India ink into the cisterna magna of a monkey in order to follow the course of diffusion. Two days later the ink had penetrated into the meninges along the whole length of the cord, over large areas of the brain stem, cerebellum, base of the cerebrum, along certain of the fissures, about half way up the lateral surfaces of the hemispheres, the choroid plexuses and the lateral ventricles. The nervous substance itself was not

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discolored, except for slight staining in the floor of the fourth ventricle.

When virus was inoculated intrathecally the earliest lesions were usually situated in the floor of the fourth ventricle. This suggested to Hurst that the penetration of the virus through the nervous tissue may occur at the ependyma of the fourth ventricle where, under experimental conditions, the virus is regurgitated at operation; but the cerebrospinal fluid does not necessarily participate in its spread through the nervous system.

EXPERIMENTAL

The work to be presented here is an outgrowth of a series of experiments conducted on the neutralizing action of immune serum upon the virus of poliomyelitis.* During the course of this study numerous discrepancies in the results were observed, and an effort was made to determine what possible factors might be responsible for such variations. Among other things, the inoculation procedure and its effect upon the results were investigated.

Experiment I

To follow the course of distribution of substances from the site of inoculation, material containing India ink was introduced into the brains of *Macacus rhesus* monkeys in the manner usually employed in our previous experiments.

Technique: The customary serum-virus mixture used in our neutralization tests was prepared. This consisted of 1.5 cc. of a Berkefeld N filtered 5 per cent suspension of poliomyelitic monkey spinal cords, mixed with 1.5 cc. of human convalescent serum. To this mixture, 1 cc. of India ink was added. After thorough mixing, the material was injected into the right frontal lobes of 4 monkeys, each receiving 0.25 cc., 0.5 cc., 1 cc., and 2 cc., respectively, with a tuberculin syringe carrying a $\frac{3}{4}$ inch, 26 gauge needle, which was inserted through a trephined opening made in the frontal bone approximately 1 cm. to the lateral right of the midline and 1 cm. anterior to the coronal suture. After 2 hours the animals were chloroformed and their brains and cords examined at autopsy. The results of this experiment are summarized in Table I.

* The details of these experiments and a review of this subject, which entail some length, will be published elsewhere.

Experiment II

A second series of 4 monkeys was inoculated in a manner similar to those in Experiment I. In this group 2 monkeys received 1 cc. and 0.25 cc., respectively, of the serum-virus-ink mixture with a $\frac{3}{4}$ inch, 26 gauge needle, and 2 others were injected with 1 cc. and

TABLE I

The Diffusion of Material Inoculated Intracerebrally (Right Frontal Lobe) Throughout the Central Nervous System, as Evidenced by the Distribution of India Ink Contained in the Inoculum

Monkey No.	Amount of mixture injected	Appearance of the central nervous system 2 hours after injection
1	cc. 0.25	There was evidence of seepage of the material from the brain substance into the subarachnoid space above the site of inoculation. Slight hemorrhage at the site of inoculation was also noted. No India ink was observed on the spinal cord (Figs. 1 & 2)
2	0.5	When the monkey was chloroformed, leakage was observed to be still taking place externally at the site of injection. On flapping back the scalp India ink was found to be deposited around the trephined opening and the surrounding area (Fig. 3). The material had diffused over the surfaces of the brain, cerebellum and spinal cord (Figs. 4 & 5). Sections at the site of inoculation showed that the injection had probably been made directly into the lateral ventricle (Figs. 5 & 6)
3	1.0	This monkey had been used in a neutralization test 2 months previously and therefore, as is often observed, had a sterile abscess in the right frontal lobe at the previous site of inoculation. On examination it was noted that although some India ink had seeped into the subarachnoid space, the bulk of the inoculum was found to be confined to the necrotic cavity on the right side (Fig. 7)
4	2.0	The entire surface of the brain and cord of this animal was covered with India ink, indicating the extensive seepage of the inoculum from the site of inoculation into the cerebrospinal fluid (Figs. 8 & 9)

0.25 cc., respectively, using a $\frac{1}{2}$ inch, 26 gauge needle. After 2 hours the animals were sacrificed and their brains and cords examined at autopsy. The findings are summarized in Table II.

From the results of these experiments it was evident that the distribution of material following intracerebral deposition varied

to some extent. In most cases, however, little of the material remained at the site of inoculation but rapidly entered the cerebrospinal fluid either by seeping backward through the path of inoculation into the subarachnoid space or via the ventricles into the spinal canal. Except for areas reached by the needle no carbon

TABLE II

Comparison of the Diffusion of Material Through the Central Nervous System after Intracerebral Inoculation with Needles of Two Sizes

Monkey No.	Amount of mixture injected	Size of needle	Appearance of the central nervous system 2 hours after injection
1	cc. 1.0	$\frac{1}{2}$ inch	The entire surface of the right frontal lobe (the side inoculated) up to the fissure centralis was covered with India ink (Fig. 10). No ink was observed on the opposite hemisphere or on the spinal cord. Sections examined at the site of inoculation indicated that the inoculum had been deposited in the brain substance at the site of inoculation below the cortex (Fig. 11), but some seeped backward and entered the subarachnoid space
2	0.25	$\frac{1}{2}$ inch	The general appearance of the brain and cord of this animal was similar to the one above, except that there was less distention of the brain tissue at the site of inoculation (Fig. 12)
3	1.0	$\frac{3}{4}$ inch	The entire cerebral hemisphere opposite to the side inoculated was completely covered with India ink (Fig. 13). Ink was also present in all of the ventricles, the base of the brain and the spinal cord (Fig. 14). The photograph clearly shows the path of the needle
4	0.25	$\frac{3}{4}$ inch	No ink was observed on the surface of the brain, but it was abundant on the surface of the spinal cord (Fig. 15). A section of the brain revealed a considerable quantity of ink in the lateral ventricle (Fig. 16)

particles were found deposited in the nervous substance itself, but the India ink adhered to the surfaces of the brain or cord. There was some suggestion that with a shorter needle and a smaller amount of material the inoculum did not as readily diffuse through the cerebrospinal fluid and reach the spinal cord. On that basis,

therefore, neutralization tests were performed to compare the results of inoculation of a large and small volume of material, using needles of $\frac{7}{8}$ inch and $\frac{1}{4}$ inch length. The $\frac{7}{8}$ inch needle was employed in order that the inoculum might reach the lateral ventricle, and the $\frac{1}{4}$ inch needle in order that the material might be deposited into the cerebral cortex. The volumes selected were 1 cc., the usual amount inoculated in our neutralization tests, and 0.25 cc., the injection of which in previous experiments had indicated a tendency toward greater regularity.

Experiment III

Technique: A set of 10 duplicate test tubes, each containing 1.5 cc. of a 5 per cent virus filtrate and 1.5 cc. of pooled human convalescent serum, was incubated for 2 hours at 37° C. and kept in the refrigerator overnight. From each of 5 of these tubes 2 monkeys were inoculated intracerebrally with 1 cc. and 0.25 cc., respectively, using a $\frac{7}{8}$ inch needle. From each of the remaining 5 tubes 2 monkeys respectively received 1 cc. and 0.25 cc. with a $\frac{1}{4}$ inch needle. Immediately before injection 0.25 cc. of sterile India ink was added to each tube in order that the dispersion of the inoculum could be followed in those animals that developed poliomyelitis. The results are summarized in Table III.

The variable manner in which material inoculated intracerebrally diffuses is again illustrated by this experiment. The extent of the distribution is apparently not entirely governed by the amount inoculated or the length of the needle employed. It is interesting to note, however, that none of the monkeys receiving material with the $\frac{1}{4}$ inch needle developed poliomyelitis, while 4 of the 10 monkeys inoculated with similar mixtures, but with $\frac{7}{8}$ inch needles, became infected.

It has been observed ⁷⁻¹⁰ that upon dilution of a neutral mixture of virus and immune serum a subsequent disruption of the virus-serum union, the so-called dilution phenomenon, takes place and the mixture again becomes infective. The results of the above experiments suggested the possibility that if some quantity of the inoculum escapes from the area of inoculation into the cerebrospinal fluid, the dilution phenomenon may occur within the animal body and thus account for the occasional infectivity of an otherwise apparently inactivated mixture.

A Comparison of the Effects of the Volume of the Needle Used to Inoculate Neutral Serum-Virus Mixtures

A Comparison of the Effects of the Volume of Inoculum and the Length of the Needle							
3/4 inch needle			1/4 inch needle				
Test tube No.	Monkey No.	Amount inoculated	Result	Test tube No.	Monkey No.	Amount inoculated	Result
1	1	cc. 1.0	Remained well	6	11	cc. 1.0	Dead, 6 days. Colitis. Cord Sections showed no evidence of poliomyelitis. India ink in subarachnoid space over the site of inoculation and over the spinal cord
	2	0.25	Paralyzed, 18 days. Ink found at site of inoculation and in lateral ventricle. None on surfaces of brain or cord		12	0.25	Remained well
	3	1.0	Remained well		13	1.0	Remained well
	4	0.25	Paralyzed, 17 days. Ink found at site of inoculation and in lateral ventricle below it		14	0.25	Remained well
	5	1.0	Dead next day of unknown cause. India ink at site of inoculation, and sub-arachnoid space above it		15	1.0	Remained well
2	6	0.25	Paralyzed, 12 days. Ink found in sub-arachnoid space over both cerebral hemispheres, also at the base of the brain and throughout the spinal cord	7	16	0.25	Remained well
	7	1.0	Paralyzed, 9 days. Ink confined to site of inoculation only **		17	1.0	Remained well
	8	0.25	Remained well		18	0.25	Remained well
3	9	1.0	Paralyzed, 4 days. Ink in all the ventricles, base of the brain and spinal cord	9	19	1.0	Paralyzed, 10 days. Ink in the subarachnoid space, base of the brain and site of inoculation
	10	0.25	Paralyzed, 5 days. Ink at site of inoculation and lateral ventricle		20	1.0	Paralyzed, 10 days. Ink at site of inoculation only **
	11	1.0	Paralyzed, 4 days. Ink in all the ventricles, base of the brain and spinal cord				
4	12	0.25	Paralyzed, 4 days. Ink in all the ventricles, base of the brain and spinal cord	10			
	13	1.0	Paralyzed, 4 days. Ink in all the ventricles, base of the brain and spinal cord				
	14	0.25	Paralyzed, 4 days. Ink in all the ventricles, base of the brain and spinal cord				
5	15	0.25	Paralyzed, 4 days. Ink in all the ventricles, base of the brain and spinal cord				
	16	1.0	Paralyzed, 4 days. Ink in all the ventricles, base of the brain and spinal cord				
	17	0.25	Paralyzed, 4 days. Ink in all the ventricles, base of the brain and spinal cord				
Controls *				Controls *			

normal monkey serum, 5 per cent virus filtrate and India ink. The India ink was confined mostly to the cavitation of the tests. (see Fig. 7).

* The controls received a similar mixture of normal monkey serum, 5 per cent virus filtrate and India ink.
 ** These animals had been inoculated 2 months previously with serum-virus mixtures and survived the tests.
 The India ink was confined mostly to the cavitation of the sterile brain abscesses often seen in monkeys examined a few weeks after intracerebral infection (see Fig. 7).

Experiment IV

To determine whether or not direct admixture with the cerebrospinal fluid would prove this point, a group of 5 monkeys was injected with 1 cc. of mixtures of virus and serum, prepared as described above, but without India ink. The inoculations were made below the dura and into the subarachnoid space above the right cerebral hemisphere. This was accomplished by surgical trephining of the frontal bone at the usual site of inoculation. The area exposed was made large enough so that sufficient assurance could be had that the brain substance was not touched upon subdural insertion of the needle. All of these animals remained well during an observation period of 2 months, whereas a group of 4 controls inoculated similarly, but receiving a mixture of the virus and normal monkey serum, all developed poliomyelitis within the usual incubation period.

Of another group of 4 monkeys, each inoculated with 1 cc. into the cisterna magna, none showed evidence of infection while the four controls became paralyzed.

Experiment V

Since during the process of an intracerebral inoculation the brain is traumatized, we decided to investigate the combined effect of direct inoculation into the spinal fluid and simultaneous brain trauma. Accordingly, a group of 12 monkeys was inoculated with 1 cc. of a neutral serum-virus mixture (4 cc. of undiluted pooled human convalescent serum and 4 cc. of 5 per cent virus suspension) intracisternally. In 6 of these monkeys, immediately following inoculation, a sterile $\frac{7}{8}$ inch needle was pushed into the brain at the usual site of inoculation and then withdrawn. The other 6 monkeys were treated in a similar manner with a $\frac{1}{4}$ inch needle. None of the 12 animals developed poliomyelitis, while the control in each group became paralyzed.

Experiment VI

Having no indication from the above experiments that the dilution phenomenon took place *in vivo*, we attempted to determine whether it would occur *in vitro*. Therefore, monkeys were injected from 3 tubes containing mixtures which consisted of equal quantities (4 cc.) of 5 per cent virus filtrate and human con-

valescent serum, 0.5 cc. of which was diluted, after the usual incubation time, with 2 cc. of normal monkey spinal fluid, giving a dilution ratio of 1:5. Twelve monkeys, 4 injected from each tube, received intracerebrally 1 cc. each of these mixtures prior to dilution with the spinal fluid, and 6 monkeys, 2 injected from each tube, received 1 cc. each of the mixtures following dilution. None of these animals developed the disease. Two controls receiving the virus and normal monkey serum and 2 others injected with virus, normal monkey serum and spinal fluid, all became infected.

DISCUSSION

Our experiments indicate that the manner in which material, following intracerebral inoculation, is distributed, resembles in many respects that noted after intrathecal inoculation, as described by Hurst.⁶ While certain variations in the course and extent of the diffusion were observed, some admixture of the material with the cerebrospinal fluid occurred in almost every instance. Generally, little of the material was found in the brain substance at the site of inoculation, except in monkeys that had received intracerebral inoculations in other experiments, in which case most of the ink was usually confined in the necrotic area of the previous site of inoculation. When larger amounts or longer needles were used, it appeared that the material more readily found its way into the cerebrospinal fluid. It may be stated, however, that the results are uncertain in so far as the final deposition of the material is concerned, but, in any event, some seepage into the cerebrospinal fluid is to be expected.

What, if any, correlation exists between the diffusion of material and its ultimate effect upon the infectivity of the neutral virus-serum mixtures cannot be answered from the data at hand. From the results of Experiment III, it appears that intracerebral inoculations with a $\frac{1}{4}$ inch needle tend towards greater regularity, since monkeys receiving the same mixtures were infected when the $\frac{7}{8}$ inch needle was used. At first, we were inclined to attribute the consistent infectivity of the neutral mixtures inoculated with a short needle to the fact that less seepage might have taken place. However, admixture with the cerebrospinal fluid seems to have no bearing upon the infectivity of these mixtures since, as observed in Experiments IV and V, direct subarachnoid or cisternal introduc-

tion or intracisternal inoculation with simultaneous brain trauma caused no reactivation of the inactive mixtures.

We sought an explanation for irregularities on the ground that the dilution phenomenon may occur. Although this has been reported to take place with a mixture of immune serum and poliomyelitis virus by Schultz and his collaborators,¹¹ we were unable to demonstrate, in our experiments, that the dilution phenomenon ensued either *in vitro* or within the animal body.

It is, therefore, difficult to state what factors may account for unexpected infections when a presumably neutral mixture of serum and virus is injected into a group of monkeys. So far as this work has been carried, there is some indication that the use of a $\frac{1}{4}$ inch needle or direct intracisternal inoculation may be an improvement over the usual intracerebral method for performing neutralization tests, but further studies on a larger scale are necessary to verify this.

SUMMARY

1. The distribution of material throughout the central nervous system of *Macacus rhesus* monkeys, subsequent to intracerebral inoculation, was studied by the injection of India ink. The experiments indicated that certain variations in the degree and extent of diffusion occurred, but in most instances the inoculum rapidly entered the cerebrospinal fluid either via the subarachnoid space or ventricles. Except for the site of inoculation, no carbon particles were found in the brain substance itself. The India ink was deposited on the surfaces of the brain or cord.

2. It appeared that when material was inoculated in larger amounts or with longer needles, it more readily entered the cerebrospinal fluid.

3. In an experiment where apparently neutral mixtures of poliomyelitis virus and immune serum were inoculated intracerebrally into monkeys with $\frac{1}{4}$ inch needles and $\frac{7}{8}$ inch needles, none of the 10 monkeys injected with the shorter needles developed poliomyelitis, while 4 of 10 monkeys receiving the mixtures with $\frac{7}{8}$ inch needles succumbed to the disease.

4. Direct inoculation of serum-virus mixtures into the subarachnoid space or cisterna magna did not render these neutral mixtures active, nor were these mixtures infective when inoculated

intracisternally accompanied by brain trauma, although controls similarly inoculated were uniformly infected.

5. Experiments devised to demonstrate the occurrence of the dilution phenomenon *in vivo* or *in vitro* were negative.

The authors gratefully acknowledge the technical assistance given by Mr. Angelo Campagna.

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DESCRIPTION OF PLATES

PLATE 45

- FIG. 1. Dorsal view of the brain of Monkey No. 21. India ink is deposited on the surface of the frontal lobe.
- FIG. 2. Ventral view of the brain of Monkey No. 21.
- FIG. 3. Monkey No. 300 with skull exposed showing external leakage following injection. India ink is deposited in the area surrounding the trephined opening.
- FIG. 4. Ventral view of brain and spinal cord of Monkey No. 300. India ink is deposited over the surfaces of the brain, cerebellum and spinal cord.
- FIG. 5. Dorsal view of brain and spinal cord of Monkey No. 300 with section through the site of inoculation showing India ink deposited in the lateral ventricle and on the surfaces of the brain and spinal cord.
- FIG. 6. Brain of Monkey No. 300 sectioned through another plane at the site of inoculation. Note the path of the needle and ink in the lateral ventricle.
- FIG. 7. Brain of Monkey No. 180 sectioned through the site of inoculation. The India ink is confined to the necrotic cavity present as a result of an inoculation given 2 months previously.
- FIG. 8. Dorsal view of brain and spinal cord of Monkey No. 88. The surfaces are heavily coated with India ink.
- FIG. 9. Ventral view of the brain and spinal cord of Monkey No. 88 showing considerable deposit of India ink.

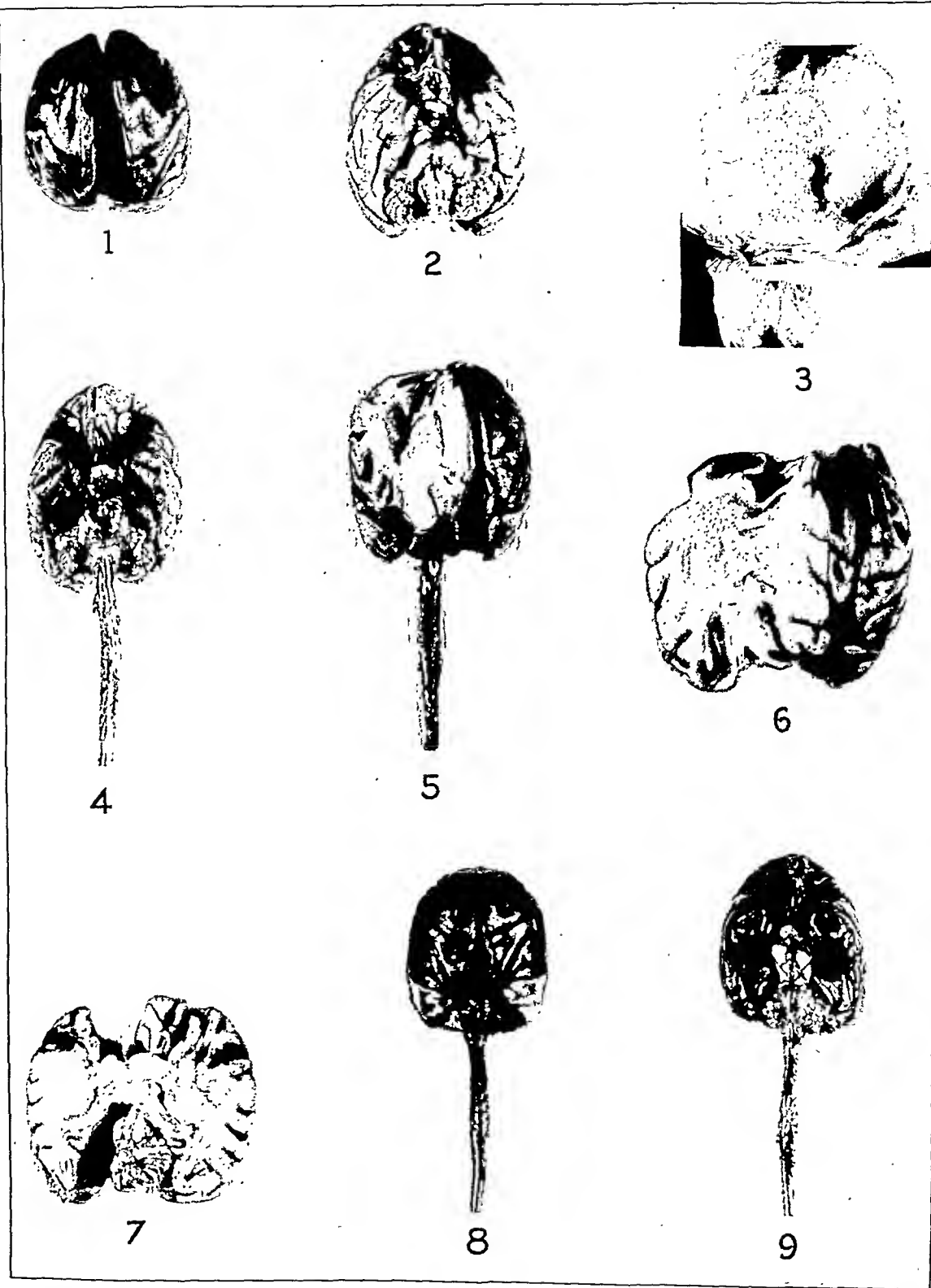
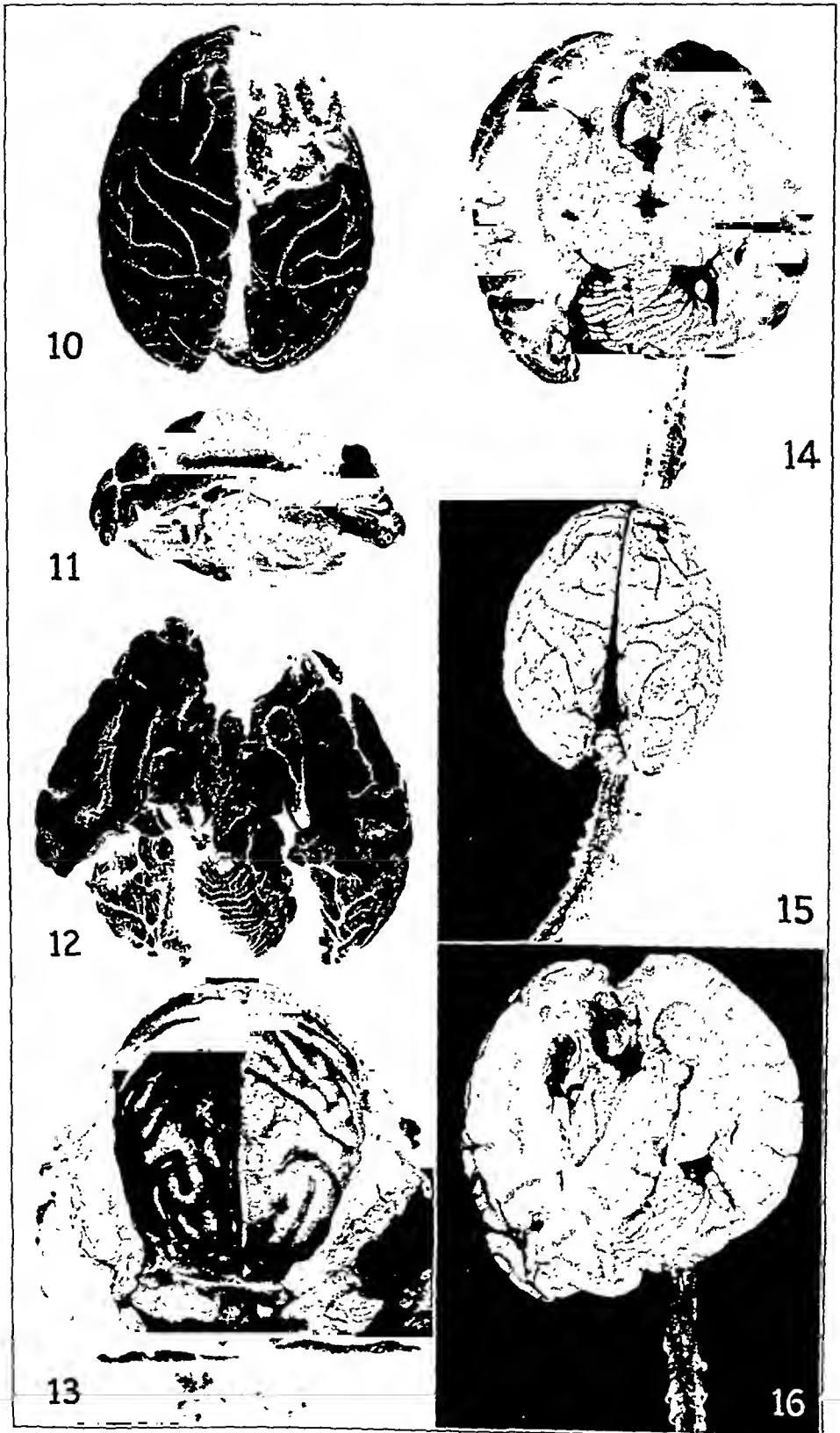


PLATE 46

- FIG. 10. Dorsal view of brain of Monkey No. 295. India ink is deposited on the entire surface of the right frontal lobe up to the fissure centralis.
- FIG. 11. Sagittal section through the right hemisphere at the site of inoculation of the brain of Monkey No. 295. The area into which the inoculum has been deposited is distended.
- FIG. 12. Brain of Monkey No. 137 sectioned through the site of inoculation. Note the India ink on the surface and in the area inoculated. Distention is also present here but to a lesser degree than that noted in Monkey No. 295.
- FIG. 13. Dorsal view of the brain of Monkey No. 225 *in situ* with dura removed showing deposit of India ink over the entire surface of the cerebral hemisphere opposite the side inoculated.
- FIG. 14. Brain of Monkey No. 225 sectioned through the site of inoculation. India ink is present in the ventricles and on the spinal cord. The path of the needle is made clearly visible by the deposit of India ink.
- FIG. 15. Brain and spinal cord of Monkey No. 207. No India ink is evident on the surface of the brain but it is abundant on the spinal cord.
- FIG. 16. Brain of Monkey No. 207 sectioned through the site of inoculation. A large amount of India ink is seen in the lateral ventricle and on the spinal cord.



A MODIFICATION OF THE MASSON TRICHROME TECHNIQUE FOR ROUTINE LABORATORY PURPOSES *

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Trichrome methods are rapidly replacing the ancient hematoxylin-eosin technique so largely used in pathology. With their use diagnosis becomes easier as a result of the topographical delimitation of the connective tissue, and the reactions of this tissue itself, such as sclerosis and the like, become at once apparent. There is no lack of good trichrome techniques in our armamentarium, but for rapid results the routine worker can but very rarely have recourse to such stains as Mallory's excellent phosphotungstic acid hematoxylin method, which necessitates treatment with iodine and bleaching with sodium thiosulphate, and a subsequent oxidation and reduction with potassium permanganate and oxalic acid. Furthermore, sections so treated only begin to show a rich color and good detail after some 6 to 12 hours of staining.

Domagk has devised a striking modification of Mallory's connective tissue stain (at the laboratories of the I. G. Farbenindustrie in Eberfeld-Leverkusen) by replacing fuchsin with a stable and exclusively nuclear stain (Kernechtrot) which is a very brilliant carmine. One does not, however, obtain the full tinctorial effects on the nuclei for some 30 minutes and, often enough, differentiation is found to be necessary. The method, excellent in itself, cannot conveniently be utilized in laboratories where there is much routine work and a need for the rapid mass production of sections. There are other modifications of the Mallory trichrome method — the gallein-orange-aniline blue and the acid alizarin blue (proposed by Petersen ¹) among them, but they are unsuited to rapid work.

Masson's ^{2, 3} trichrome method remains one of the best, combining as it does the most precise of hematoxylin (Heidenhain's iron hematoxylin) with a reliable cytoplasmic stain that gives a wealth of detail (acid fuchsin with ponceau de xylin), and a very selective stain for connective tissue (light green or aniline blue). This

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method requires mordanting with ammonio-ferric alum for 24 hours, followed by staining with Regaud's hematoxylin (the formula based on Heidenhain's) for a similar length of time. One may shorten the process to an hour by mordanting for 30 minutes and staining a like period at 50° C. If one shortens the process still further, however — say to 5 minutes in each solution — the stain becomes neither precise nor stable; the nuclei soon fade out, even during the finishing of the sections, being replaced by fuchsin. Furthermore, they must be differentiated in picric acid alcohol and this process should be followed under the microscope to avoid over-differentiation. When one must be staining a hundred sections at a time, this very excellent trichrome method becomes difficult of application. Although fine in itself, it was not devised for busy pathologists.

I have, therefore, modified it so that it might be made more applicable to routine needs. The Heidenhain-Regaud hematoxylin has been replaced by the ferric trioxymatein of Hansen; the other elements of Masson's stain have been diluted to avoid areas of concentration and to give more transparency to the sections, as well as obviating the evils of long exposure to a stain. Phosphomolybdic acid has been replaced by phosphotungstic and the strength of this increased to 5 per cent in order to shorten the time element. Finally, a third cytoplasmic stain (orange G) has been added to accentuate the erythrocytes, to increase the yellow tones and to afford a supplementary color. The colloid of the thyroid, for instance, may take on a green color, or various shades of red with the fuchsin, or it may come out a pure orange. This probably depends upon varying chemical characteristics of the colloid under varying metabolic circumstances. With this modification one thus obtains a good stain that resembles Masson's, without always showing all of its characteristics, completed within a period of from 20-40 minutes. Naturally, the time required for deparaffinization, and so on, is not reckoned in. In appropriate cases the procedure may be shortened to even 10 or 12 minutes.

Hansen's ferric trioxymatein is a stable precise dye of the color of lithographic ink, sometimes verging on the sepia. Of itself, it gives a wealth of detail (granules, cilia, fibrillary structures, cuticulae of epithelium, and even spirochetes). It does not obscure the connective tissue, particularly after formalin fixation, where

Heidenhain's hematoxylin produces grayish areas that cannot be decolorized. It may be differentiated, if needs be, in weakly acidulated water or alcohol, but this is not usually necessary, the various tissue elements appearing in gradations of gray to black through the brick red background of the fuchsin-ponceau, provided that this has been sufficiently diluted. In short, it may be rendered a purely nuclear stain by the addition of sulphuric acid to the tri-oxyhematein solution. In this way one need not fear overstaining which is important in laboratories where one has insufficient leisure to dedicate much time to individual sections.

It is for the same reason that the cytoplasmic stains of Masson's method have been diluted. The tinctorial effects are obtained in a minimal period of time, the dilution prevents overstaining and affords acceleration of the process; finally, the sections show increased transparency and have more agreeable tonalities, both under the microscope and on the projection screen. This variant may be used on any material fixed in any of the usual fluids, provided one observes the usual formalities — iodine and sodium thiosulphate after Zenker's fixative, or alcohol and lithium carbonate after Bouin's, and so on. It may be used on paraffin sections, from tissue fixed in neutral formalin or alcohol-formalin, but the orange G tends to stain a little more irregularly and diffusely in these than in those fixed in Zenker's or Bouin's fluids.

This method has been adopted as the routine stain in the laboratories of the Department of Surgical Pathology of the New York Hospital and Cornell University Medical College. It has proved to be a vast improvement over the trichrome light green method used with success for the past 5 years. Its advantages are: (1) increased transparency with consequently increased histological detail, particularly shown in muscular tissue; (2) much more precise nuclear detail and no replacement of the nuclear stain by the red elements; details of mitotic figures are beautifully brought out; (3) no "piling up" of the light green in connective tissue or mucus, hence no obscuring of connective tissue details; and (4) better color values for the purposes of microphotography.

METHOD OF PROCEDURE

1. Stain deparaffinized sections taken out of water in Hansen's iron hematoxylin for 1–5 minutes. The dye may be used pure, or it

may be acidified with 2 parts of 2 per cent aqueous sulphuric acid to 8 parts of the dye. Longer staining does no harm, as it can not overstain the tissue if it has been acidulated. The solution may be used repeatedly, but it must be filtered before using. It must be sepia black; if it becomes greenish a new supply should be made up. It keeps for approximately 6 weeks in ordinary use.

2. Wash the sections at the tap as long as yellowish brown clouds come off in the water. After 5 minutes the nuclei should be a rich black.

3. Stain in Masson's fuchsin-ponceau mixture, diluted 10 times with water acidulated to 0.2 per cent (1:500) with acetic acid, for 5 minutes or more.

4. Rinse in distilled water acidulated in the same fashion. If the city supply be not too alkaline, tap water may be used. Usually a few drops of glacial acetic acid in tap water will work well.

5. Treat for 15 seconds to 30 minutes with phosphotungstic acid orange G. A few minutes usually suffice amply.

6. Repeat the rinsing as in Step 4.

7. Stain for 5 minutes in Masson's light green solution diluted 10 times with water acidulated as above.

8. Rinse as in Step 4 for 5 minutes to eliminate the phosphotungstic acid and to differentiate the various color tones.

9. Dehydrate in the usual manner with ascending percentages of alcohol, clear in xylol and mount in balsam.

Results: The nuclei are black to brownish black; the cytoplasm is brick red (certain granules stain more golden); erythrocytes are yellowish vermilion to orange; collagen and mucus bluish green. Other structures appear in various shades of gray superimposed upon the reddish background.

Variants

(A) If time is no object, one may prolong the hematoxylin stain to 15 minutes, differentiate in 2 per cent aqueous sulphuric acid until one obtains the desired accents on elements to be brought out, and then wash in water and proceed with the rest of the stain.

(B) Fuchsin-ponceau may be replaced by azophloxine (Hollborn), which resembles eosin but does not "pile up" or overstain. Its tones are warmer and the erythrocytes are very selectively demonstrated in shades of cardinal red to salmon pink. Further-

more, it is extremely stable and has been exposed to sunlight during an entire summer without deterioration. A 0.05–0.1 per cent solution is prepared in water containing 2 drops of acetic acid to 100 cc. of water. The solution should have thymol, or other disinfectants added to it to prevent the formation of molds. It stains almost instantaneously, but sections may be left in it for 2–5 minutes. Sections stained with this dye remind one of those prepared by Prenant's trichrome method which was, in the days before Masson's method appeared, one of the most employed techniques in French laboratories, or those under French influence.

Formulas and Preparation of Staining Solutions

1. Hansen's Trioxyhematein

Solution A: Dissolve 10 gm. of ammonio-ferric alum (amethyst crystals) and 1.4 gm. ammonium sulphate in 150 cc. of distilled water, warming gently. This may also be done in the cold.

Solution B: Dissolve 1.6 gm. of hematoxylin in 75 cc. of distilled water in a porcelain dish over the flame.

When the two solutions have thoroughly cooled, pour *Solution A* into *Solution B* (never *vice versa*!); the mixture, at first brown, changes to blue and then to deep violet. The color changes may be checked up by placing drops of the mixture on filter paper from time to time. While pouring A into B the containers should be constantly agitated to ensure even mixing. When the color has become violet heat the mixture cautiously to avoid overoxidation and do not wait for the boiling point to be reached if the test drops on the filter paper are a brownish black, or sepia color. Under no circumstances boil for more than 30–60 seconds. Chill the mixture abruptly, after the end-point of the reaction has been reached, by floating the dish on cold water. When bottling, fill up to the neck to avoid leaving an air space at the top that might cause superoxidation. Bottles of Pyrex, or some such glass, are better than those of ordinary glass, which tends to be alkaline. The final solution should be sepia black; if olive green it will stain poorly. The greenish tinge indicates overoxidation. The solution may be restored to its original sepia color by adding 10 per cent oxalic acid, drop by drop, until the desired tone is obtained. The solution keeps for a long time. In order to render the stain strictly and exclusively

nuclear, 2-4 parts of 1 per cent aqueous sulphuric acid should be added to 8 parts of the dye.

2. *Masson's Fuchsin-Ponceau (Dilute Formula)*

Ponceau de xylidine (Krall or Hollborn)	0.2 gm.
Acid fuchsin (acid rubin) (any good brand)	0.1 gm.
Distilled water with 0.2 per cent acetic acid	300 cc.

If the original formula is kept in stock, it should simply be diluted 10 times.

3. *Phosphotungstic Acid Orange G*

Phosphotungstic acid	3-5 gm.
Orange G (Hollborn "standardized")	2 gm.
Distilled water	100 cc.

4. *Light Green*

Light green (Lichtgrün)	0.1-0.2 gm.
Distilled water with 0.2 per cent acetic acid	100 cc.

NOTE: Since submitting this manuscript, several thousand sections have been stained in our laboratory. During this time we have perfected the method and simplified it still further.

Weigert's iron hematoxylin nuclear stain gives fine results, often better than those obtained with Hansen's hematoxylin, and its preparation is far simpler. In order to avoid the necessity for differentiating with acid, the dye should be diluted with an equal volume of 50 per cent alcohol, as in Petersen's method. With this there is no overstaining within 5-10 minutes. After staining wash the slides with 50 per cent alcohol acidulated with 0.1 per cent hydrochloric acid. It is well to increase the amount of hematoxylin in the formula from 1 to 1.25 gm. in 100 cc. of alcohol. The mixture of hydrochloric acid and ferric chloride, as given in the formula, remains unchanged. The stain will keep for 1 day only and must therefore be made up fresh each day. Harris' hematoxylin, if overstained slightly (5-10 minutes), gives excellent results and it is not necessary to differentiate the stain as the acids that are used in the other stains later extract a certain amount.

Doctor Foot has found it advisable to add the orange G to the mixture of ponceau and acid fuchsin, in which case the sections may be mordanted in phosphotungstic acid with or without the addition of orange G. This dye is taken by the erythrocytes, keratin, sheaths of peripheral nerves (neurokeratin?) and the mature colloid of the thyroid, which transforms the stain from a trichrome to a tetrachrome. Following Zenker's and Bouin's fixatives, Hollborn's orange G is preferable to "certified"; following the alcohol-formalin fixative the reverse is true. The cytoplasmic stain, in this case, is made up as follows:

Ponceau-acid fuchsin, stock mixture	10 cc.
Orange G, 2 per cent aqueous solution	10 cc.
Distilled water	80 cc.

Personally I prefer the cytoplasmic stain in which the ponceau is reinforced with azophloxine to demonstrate eosinophilic granules and erythrocytes. The formula is as follows:

Masson's Ponceau-Fuchsin Stock Solution

Ponceau de xylydine, 1 per cent aqueous solution	3 parts
Acid fuchsin, 1 per cent aqueous solution	1 part

Staining Solution

Above mixture	5-10 cc.
Azophloxine, 0.5 per cent aqueous solution	2 cc.
Distilled water, acetified	88 cc.

The orange, in this instance, is introduced into the stain through the phosphotungstic acid bath.

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USEFUL METHODS FOR THE ROUTINE EXAMINATION OF BRAIN TUMORS *

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The diagnosis of brain tumors has become increasingly complex with the added complexity incidental to the more accurate classifications resulting from the work of the Spanish school along these lines. Where "glioma" formerly sufficed for a diagnosis of neuroglial neoplasms, we must now distinguish between tumors arising from spongioblasts, astrocytes, oligodendroglial and ependymal cells. In routine work the making and impregnating of frozen sections is irksome, unless one be busied with little else. The technician must be highly trained and capable of using discrimination; otherwise the pathologist is constrained to do his own technique. In a laboratory where other routine material is coming through uninterruptedly this is often, if not usually, difficult of accomplishment.

It is the purpose of this article to set forth the routine method adopted in this laboratory, whereby any competent technician may prepare paraffin sections for the diagnosis of brain tumors along with the daily routine. It has been in use for about five years now and has been very satisfactory. Our diagnoses, checked against occasional autopsy diagnoses of our neuropathologist, have been in perfect agreement with his, which were made on frozen sections impregnated by the del Río Hortega method. Our procedure is as follows:

FIXATION

It is important to employ a number of fixatives when dealing with brain tumors; if one does not give good results, another will, so that three or four should be used, depending upon the amount of material to be fixed. If the specimen be very small, one should choose that fixative most likely to succeed.

Formalin-Alcohol: This is the routine fixative for the daily work in our laboratory. It consists of 10 parts of strong formalin to 90 parts of 95 per cent alcohol. Blocks of tissue not thicker than 4 mm. are placed in this solution for 7 to 8 hours. It removes a

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good deal of the lipins, clears the background and gives excellent results. Aqueous neutral 10 per cent formalin is not to be recommended for these purposes.

Bouin's Fluid: This is used in the formula recommended by Masson: water 30 parts, strong neutral formalin 10 parts, 2 per cent trichloroacetic acid (instead of glacial acetic) 2 parts, and picric acid to saturation. Thin blocks of tissue are fixed in this for 12 to 24 hours.

After fixation the tissues should be washed in water and then treated for at least 2 hours with 80 per cent alcohol saturated with lithium carbonate.

Cajal's Formalin-Ammonium Bromide: This is made up of strong formalin 15 parts, ammonium bromide 3 parts, and water to make 100 parts. It is the classic fixing fluid for tissues to be impregnated by the various silver and gold methods, but it is not generally realized that it gives superior results in trichrome sections as well.

Zenker's Fluid: The regular formula, which need not be given here, is used. Sections from Zenker-fixed tissue should be treated with weak alcoholic iodine for a few minutes, and then with a 0.5 per cent aqueous solution of sodium thiosulphate, in the usual manner, to remove the mercury precipitate before staining.

In practice, the first three methods of fixation usually suffice; the last may be held in reserve if Zenker-fixed material is needed later on.

STAINING METHOD

The Goldner modification ¹ of the Masson trichrome technique has become our routine stain and it gives excellent results in the case of brain tumors which, in most instances, may be diagnosed through the use of this method alone, without recourse to silver impregnations, although the latter are always used for purposes of confirmation. Tissue fixed in the fluids just enumerated is embedded in paraffin and cut in the usual manner.

Staining Solutions

1. Hansen's Trioxyhematein

Dissolve 10 gm. of ammonio-ferric alum (amethyst crystals) and 1.4 gm. of ammonium sulphate in 150 cc. of distilled water,

warming gently over a flame. Next 1.6 gm. of hematoxylin are dissolved in 75 cc. of distilled water in a porcelain dish with a capacity of 250 cc. or more, over a flame. When the two solutions have cooled, they are mixed by pouring the former into the latter (never the reverse!), the container being agitated continuously to ensure perfect mixture. The color changes from brown to blue and then to violet. When the violet stage has been reached, the mixture is cautiously heated until test drops on filter paper are sepia black. Never boil for over 1 minute and do not wait for the mixture to boil if the color is arrived at before the boiling point is attained. Cool the mixture suddenly by floating the dish in cold water, to prevent overoxidation. A greenish tinge indicates this. The addition of 10 per cent oxalic acid, added drop by drop, will restore the desired color by reduction. The stock solution keeps a long time, but should be tightly stoppered to avoid oxidation. In order to render the stain selectively nuclear, 2 to 4 parts of 1 per cent aqueous sulphuric acid should be added to 8 parts of the stock solution.

(1 a.) Harris' hematoxylin made according to the usual formula may be substituted for iron hematoxylin. It works equally well, but the sections should be overstained (say 5 minutes), as the picric acid and acetic acid used subsequently tend to take some of it out of the tissue.

2. *Masson's Fuchsin-Ponceau, Modified with Orange G* (*Dilute Formula*)

To 300 cc. of distilled water acidulated with 0.2 per cent acetic acid, 0.2 gm. of ponceau de xyloidine (Krall) or xyloidine ponceau (Hollborn), and 0.1 gm. of any good brand of acid fuchsin, are added. We have found it advisable to shift the orange G from the phosphotungstic acid bath (as proposed by Goldner) to this solution, adding 0.2 gm. of the dye. It is more convenient to make up a stock solution of the original strength (2, 1, and 2 gm.) and dilute it 10 times with acetified water, as the weighing of tenths of a gram is troublesome.

3. *Phosphotungstic Acid*

This replaces the original phosphomolybdic acid in Masson's formula. It should be made up in 3-5 per cent strength.

4. *Light Green*

This is a 0.1 per cent solution of light green (Lichtgrün) in acetified distilled water (0.2 per cent acetic acid).

Procedure

Sections are stained in the iron hematoxylin for from 1-5 minutes, in Harris' solution for 5 minutes. With sulphuric acid added to the iron hematoxylin, it cannot overstain and may be used repeatedly, filtering frequently, until it becomes greenish, when it should be replaced. The sections are then washed at the tap until yellowish brown clouds of dye no longer come away in the case of iron hematoxylin, or until the sections are blue in that of Harris' hematoxylin.

The sections are next stained in the fuchsin-ponceau-orange G mixture for 5 minutes or longer and are then rinsed in acetified water. If the city supply be not alkaline, tap water may be used.

The slides are next immersed in the phosphotungstic acid solution for a few minutes and rinsed in acetified water, after which they are stained in the light green solution for 5 minutes and treated with acetified water for a like period to eliminate any remaining phosphotungstic acid and to differentiate the color tones. They are then dehydrated and mounted in balsam in the usual manner.

The procedure is really not at all formidable and the technician soon becomes accustomed to its different steps.

SILVER IMPREGNATION

Solutions

1. *Pyridine-Glycerin*: This is 2 parts of pyridine to 1 part of glycerin.

2. *Impregnating Fluid*: Into 10 cc. of a 10.2 per cent silver nitrate solution, strong ammonia is dropped from a dropping-bottle until the resulting precipitate is almost dissolved; the process should best not be carried to the "water-clear" stage, but the solution should be slightly turbid. To this, 10 cc. of 3.1 per cent solution of sodium carbonate in distilled water is added and the mixture made up to 100 cc. with distilled water.

3. *Reducer or Developer*: This is made up of 3 cc. of 1 per cent

sodium carbonate (buffer) in distilled water, 1 cc. of strong formalin, and distilled water to make 100 cc.

4. *Toning Solution*: This is a 1:500 solution of gold chloride in distilled water.

5. *Intensifier*: Oxalic acid 2 gm., strong formalin 1 cc. and water to make 100 cc.

6. *Fixing Fluid*: A 5 per cent solution of sodium thiosulphate, or "hypo."

7. *Counterstain (van Gieson)*: To 10 cc. of a 1 per cent solution of acid fuchsin add 90 cc. of a solution of picric acid in water, saturated at room temperature.

Procedure

1. Sections are deparaffinized and carried into pyridine-glycerin for 24 hours. (Keep under hood to avoid unpleasant odors and possible headaches.)

2. Rinse in 95 per cent alcohol followed by distilled water.

3. Impregnate in the silver solution for $2\frac{1}{2}$ hours at 40° C. (If the solution is cold at the start, allow 15 minutes for it to warm up, making the time $2\frac{3}{4}$ hours.)

4. The sections are next washed in distilled water.

5. Reduce for 5 minutes in the developer, until the sections turn dark brown.

6. Wash in tap water.

7. Next tone for 5 minutes in the gold chloride solution.

8. A wash at the tap follows.

9. Intensify in the intensifier for 5 minutes.

10. Rinse in tap water.

11. Fix in the sodium thiosulphate solution to remove surplus unreduced metal.

12. Wash again in tap water.

13. The sections may then be counterstained in the van Gieson solution, but this is optional.

14. Dehydrate and clear sections and mount in balsam.

Here again, the rather formidable schedule proves to be less so as one becomes used to it. The silver solution may be made up in bulk and kept in a dark ice-box for use as needed. If a precipitate forms it can usually be redissolved by gentle shaking, or it may be filtered out.

MODIFIED RAMON Y CAJAL METHOD

It often happens that one would like to ascertain something concerning the nervous tissue proper in connection with the infiltration and invasion of brain tumors. After experimenting with several methods we have found that a modified block impregnation, as originated by Ramon y Cajal, gives the best results.

Procedure

Blocks of tissue not over 4 mm. in thickness are fixed in 25 per cent aqueous chloral hydrate for 24-48 hours, the latter time being preferable. This method requires this special fixation, which should be noted. The blocks are then rinsed in distilled water for a few seconds and blotted with filter paper to remove the excess water. They are next transferred to a mixture of 95 per cent alcohol and 4 drops of ammonia for 24 hours, after which they are rinsed for a few minutes in distilled water.

Impregnate with a 1.5 per cent aqueous silver nitrate solution at 37° to 38° C. for a week in the dark. If the solution becomes yellow, as it may after 30 to 60 minutes, it should be renewed with fresh solution, after which it will remain colorless.

The blocks are then developed with a mixture of 2 gm. of pyrogalllic acid, 8 cc. strong formalin, and 100 cc. of distilled water for 12 hours, or at least overnight. Then wash for 3 to 4 hours in distilled water and transfer to 80 per cent alcohol. The alcohol will turn yellow but this has no significance. The blocks are then carried through whatever fluids one may desire for paraffin embedding. Sections cut from the paraffin blocks need no further treatment save deparaffinization; it is well to discard the first few sections cut, as the periphery of the blocks is usually over-impregnated.*

RESULTS

By preparing sets of sections from tissue fixed in alcohol-formalin, staining one with the Masson-Goldner** trichrome

* We are indebted to Dr. José Nonidez of our Department of Anatomy for calling to our attention this excellent method.

** If Harris' hematoxylin has been used, the nuclei will stain the usual purplish color. With orange G added to the ponceau-fuchsin, we find that the orange dye is specifically selective for myelinated nerve sheaths which, in this case, take no other color but come out bright orange-yellow. Fibrin shows more affinity for the

method, and impregnating the other with silver, one may have a reasonably accurate diagnosis in a few days time. The sections from material otherwise fixed will then be ready in a day or two and may be used for checking up on this diagnosis. The Ramon y Cajal sections will, of necessity, not be ready for a couple of weeks. It so happens that formalin-alcohol is one of the most satisfactory fixatives, so that the early sections are often just as good as those that follow later.

Masson-Goldner Sections: Brain tumors stained by this method show sepia to black nuclei, pink neuroglia, red cytoplasm and cell processes, green connective tissue about the vessels and meninges, and coral red to orange erythrocytes. Medullated and non-medullated nerve fibrils stain more densely red than the neuroglia and may even come out a vermilion color. Sections from tissue fixed in aqueous formalin (which is not recommended) may show a greenish coloration of the neuroglia, which is misleading. In edematous tumors the coagulated serum sometimes stains a greenish hue. Fibrin stains brilliant vermilion to orange. The effects obtained with this stain are excellent and bring out the finer histological details of the astrocytes and other cells. It should be repeated that formalin-ammonium bromide gives very fine results with this trichrome method.

Silver Sections: It is not claimed that this will succeed with normal brain tissue, for it is not particularly satisfactory in demonstrating normal astrocytes, and so on. On the other hand, the less mature cells of tumors, even those of well differentiated astrocytomas and oligodendrogliomas, are well brought out; their processes are dark purple or brown, the nuclei are black, and the cytoplasm varies from warm brown to black or purplish. Vascular reticulum is usually black, but the impregnation is not directed toward the demonstration of this tissue, which is better shown in sections that have been treated with potassium permanganate and oxalic acid instead of pyridine-glycerin.

As has been said, the sections from blocks fixed in a certain fluid may not be very fine, but in this case those from one of the others will be quite satisfactory; often all of them come out equally well.

ponceau. The orange G also stains keratin selectively and shares in the staining of muscle and erythrocytes with the red dyes, in the case of sections made from tissues other than nervous tissue.

With a neutral alcohol-formalin, formalin-ammonium bromide (which suppresses the impregnation of reticulum) and an acid Bouin's fixative one is practically certain of getting sections that will make accurate diagnosis possible.

The figures in the plate demonstrate results of silver impregnations on various tumors (Figs. 1, 2 and 3).

Ramon y Cajal Sections: These come out in the old gold and black color scheme familiar to those who have used Levaditi's method. The black, however, is confined to the neuraxons. The nuclei are deep brown. The collagen and reticulum do not stand out very prominently. This is of great importance, for there can then be no confusion between reticulum and nerve fibers.

The general effects are illustrated in Figure 4, which shows surviving neurons in a brain infiltrated by glioblastoma multiforme.

DISCUSSION

We have thus far diagnosed astrocytomas, polar spongioblastomas, glioblastomas of the multiform type, oligodendrogliomas and a glioblastoma of sympathetic origin arising from the suprarenal region. This last tumor would have passed as an unusual carcinoma, in fact it did until silver impregnations demonstrated the morphology of the multipolar astrocytes it contained. Their processes were practically unnoticeable in the hematoxylin-eosin sections, but better shown in the trichrome sections, which led us to try silver impregnation. In this they came out beautifully.

There is no reason why the combination of the methods set forth in this paper should not be equally good for ependymomas and those tumors of the more immature type — the so-called medulloblastomas and neuroepitheliomas. We have found them excellent in the case of meningiomas. Naturally, they are also applicable to tumors of peripheral nerves, where they give splendid results and afford a means for accurate diagnosis.

NOTE: Miss Anna Mary McDowell has been of great assistance in the working out of these methods in our laboratory.

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DESCRIPTION OF PLATE

PLATE 47

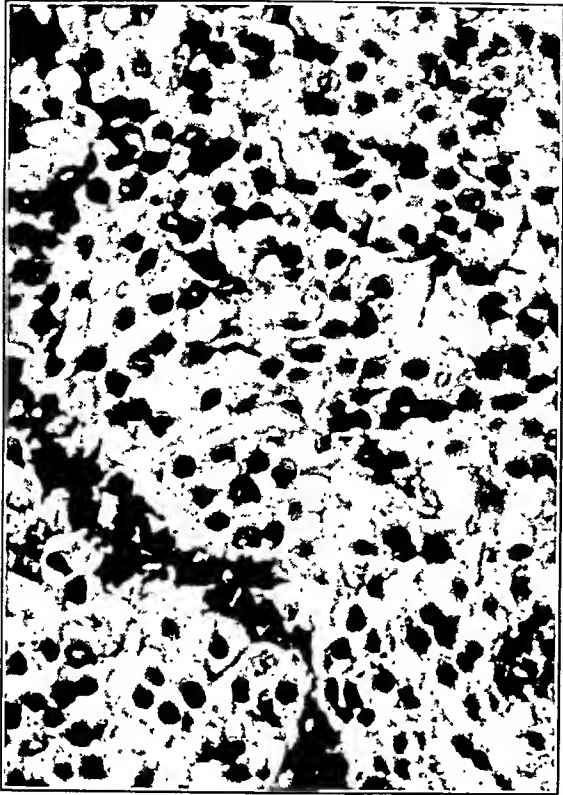
The photomicrographs were taken at a magnification of $\times 480$, using a K 3 filter instead of the usual green filters employed with hematoxylin-eosin sections.

FIG. 1. A rather protoplasmic form of oligodendroglioma. Bouin fixation.

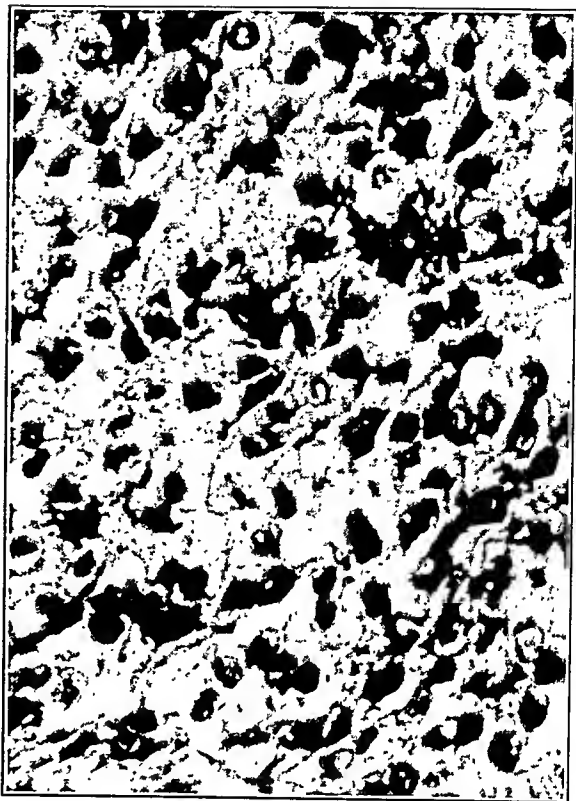
FIG. 2. A polar spongioblastoma. Alcohol-formalin fixation.

FIG. 3. A glioblastoma multiforme. Ramon y Cajal formalin-ammonium bromide fixation. These three figures are from sections impregnated by the pyridine silver method.

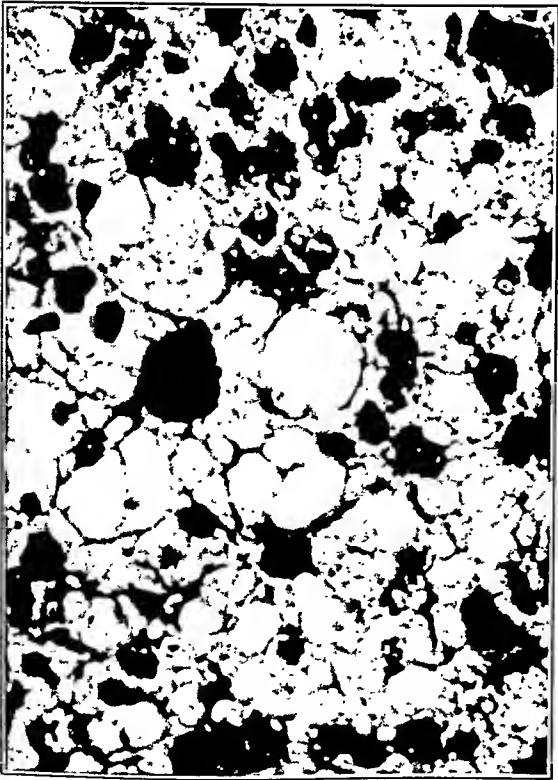
FIG. 4. A glioblastoma multiforme invading brain tissue, little of which has survived; only the neurofibrils have withstood the attack by the tumor cells. Chloral hydrate fixation.



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DEGENERATIVE ARTHRITIS *

A COMPARISON OF THE PATHOLOGICAL CHANGES IN MAN AND EQUINES

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It is now known that many animal species suffer from joint diseases of both known and unknown etiology and that some of these are similar in character in all species studied. In a previous paper ¹ we reported the results of an investigation which showed that a very prevalent condition among Army horses and mules in Panama, which had been considered a form of osteomalacia, and which caused a large amount of lameness, was in reality degenerative arthritis of undetermined etiology. It was also indicated that the condition in the horses and mules was very similar to the degenerative type of arthritis in man. We present in this paper results of a comparative study of the affection in man and the equine species.

Arthritis may be divided into two general types, inflammatory and degenerative. Both include diseases of known etiology. In the inflammatory group there are the specific infections, of which tuberculosis, gonorrheal and streptococcal arthritis are examples in man. In lower animals the arthritis of pyosepticemia or "joint-evil" due to *Shigella viscosa*, *Salmonella abortivo-equinus*, *Escherichia coli*, or streptococci, is the outstanding example of this type. In the degenerative group Charcot's arthropathy seen in syphilis ²

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and also following traumatic nerve destruction ³ is the outstanding lesion of known etiology. In addition to these there are inflammatory arthritides sometimes resulting in ankylosis, of which the etiology is obscure though they are generally designated as "rheumatoid" or atrophic, and degenerative lesions of unknown etiology which, as they usually present clinical symptoms only in the later stages when compensatory bone production occurs, are then called hypertrophic arthritis.

This paper is based on 60 specimens from man and one or more joints from each of 54 horses and mules destroyed because of disabling lameness. Most of the specimens from man were from the knee and, with the exception of one or two from the surgical clinic, they were obtained at autopsy.

The various types of pathology of diseased joints in man have been described by Nichols and Richardson,⁴ Allison and Ghormley,⁵ Parker *et al.*,^{6,7} and others. The pathology is also presented in the papers of Keefer *et al.*,⁸ and in the review prepared at the request of the American Committee for the Control of Rheumatism, by Hench *et al.*⁹ Degenerative changes in the joints of horses and mules are described in the papers of Williams, Fisher and Udall,¹⁰ Hare,¹¹ Bennet and Bauer,¹² Kintner and Holt¹³ and others. Parker's papers describing the changes in the knee joint with advancing age ⁶ and in rheumatoid arthritis ⁷ serve to clarify the essential difference in the pathology of these conditions. The first paper described degenerative arthritis, the second the essentially inflammatory nature of rheumatoid arthritis. Although publications prior to Parker's described many of the changes of degenerative arthritis, none are as definite as his, and the others at times are confusing because of the inclusion of pathological features of other conditions.

Certain changes have been observed in the material on which this paper is based that were not described by Parker and concerning some of these we have found no references. These studies appear to indicate rather clearly the sequence of events in the joint changes so the lesions will be described in the order in which they apparently occur, or rather in the order of their severity, for some of them may not be progressive.

In order to define the pathological changes the normal histological structure of the joints in man and in horses and mules will

be briefly described. Starting at the joint surface there is, in young animals and children, a layer of cells whose nuclei are roughly parallel with the surface and which appears to be continuous with the surface layer of the synovial membrane at the margin of the joint. In adults this layer of cells cannot be made out on the bearing surface of the cartilage but in the same position there is a membrane-like condensation of protoplasm which limits the more or less homogeneous ground substance between it and the underlying cartilage matrix (Fig. 1). It appears, as has been stated by others, that there is no synovial membrane over the central bearing surface of the joint cartilage, yet when loss of substance occurs on apposed surfaces in degeneration of the cartilage, so that such surfaces are no longer in contact, a membrane may form over them which appears to be synovial in character. There is no traceable connection between these membranes and the peripheral synovia except where ulcers are near or at the joint margin (Fig. 2). Beneath the peripheral membrane of the normal joint surface the homogeneous cartilage begins and extends to the calcified matrix overlying the cortical bone. Near the periphery the cartilage cells have their long axes more or less parallel to the surface. As the distance from the periphery increases the axes assume more and more a vertical position with relation to the bone cortex, and especially in horses and mules, tend to be arranged in rows at right angles to the subchondral bone (Fig. 3). In man, even at ages prior to complete ossification of the epiphyses, the cells are arranged much less regularly and are more often found in groups of nuclei not all of which have independent capsules (Fig. 1). The cells, and especially the nuclei, are larger and apparently fewer in number near the calcified matrix in both man and equines.

In so far as we can determine from a few specimens, prior to the completion of epiphyseal ossification no calcium deposit is found about the cartilage cells in man or equines, as indicated by staining reactions in frozen sections of joint cartilage which has not been decalcified. However, as early as the 24th year in man, in this series, frozen sections stained by hematoxylin show zones of deep blue staining granules about the cell capsules. These are largest near the calcified matrix, decreasing to practical absence near the joint surface. These calcific zones have been found in

apparently normal cartilage at all older ages examined. Similar deposits were found in the normal cartilages of full-grown horses but not in those of a 10 months fetus. This finding suggests a change in the chemical composition of the cartilage about the time when ossification is complete.

The cartilage terminates abruptly at the calcified matrix. This is a layer of material having nuclei of the cartilage type and containing calcium which often appears in wavy striae parallel to the bone cortex, the stria joining the cartilage usually being the most densely stained with the blue of hematoxylin. Normally this layer is distinct at its junction with the cartilage, but projects into the bone cortex in irregularly shaped papillary-like masses which blend with the underlying cortical bone. Calcium appears to be retained in this matrix after decalcification processes have completely removed it from the normal bone beneath, in so far as staining reactions reveal its presence (Fig. 3).

Beneath the calcified matrix is the cortical bone, the character of which varies with the species, size and activity of the animal and differs in each bone and in different parts of the same bone. In general, large surfaces which bear the stress more or less evenly show little thickening or condensation of the trabeculae of the cortical bone. The central portions of the femoral condyles are examples. Localized areas where the subchondral trabeculae form compact bone with few marrow spaces occur at points of maximum stress, as in the grooves and on the apices of the ridges of the tibial tarsal bone and the tibia of horses and mules.

In judging as to the condition of the bone, therefore, these variations must be remembered, as must also the natural difference in bony structure of larger or smaller animals and also variations secondary to physical activity.

In fetal cartilage no definite line of demarcation can be made out between that portion which will remain cartilage and that which will be transformed into bone. Most of the blood vessels loop back at about normal cartilage depth from the periphery but occasional vessels approach nearer the joint surface. After the bone cortex is defined and the calcified matrix is laid no vessels are found in the cartilage, but in all types of cortex, from the least to the most dense, vascular tissue is found projecting from the marrow spaces into or even through the calcified matrix. These

projections are more numerous in cortical bone in which there is little peripheral condensation of the trabeculae (Fig. 3).

It appears to us that variations in density of the bone cortex have been misconstrued as changes due or secondary to degenerative arthritis, the lesions of which are prone to occur at just those points of stress where normally the bone is more dense. With these normal conditions and variations in mind the pathological lesions of degenerative arthritis, as observed by us in man and in horses and mules, will be described.

The mildest lesion seen consists of parallel grooves running in the direction of motion of the joint surfaces. These are relatively infrequent in horses and mules and rare in man. In the former they are found in the hinge joints like the tibiotarsal; in man on the patella, especially on the middle facets. In the equine species this may be the only type of lesion of a joint and it involves both joint surfaces, while in man other changes have always been present and the patellar cartilage alone may be affected.

Grossly the lesion consists of grooves of varying depth, the lateral margins appearing slightly raised. There is little change in color except in the deeper ones in equines in which the depth of the depression in some areas is dark red in color, the base being so thinned that the vascular underlying bone shows through. Hare,¹¹ in his study of this type of lesion, found that it consisted of ridges and grooves, the ridges of one of the apposed surfaces fitting into the grooves of the other. This we have been unable to demonstrate in our specimens. In fact, the depth of the groove often is made up of less dense tissue than the surrounding cartilage, indicating lack of pressure in this area. In man only shallow grooves have been seen (Figs. 4 and 5).

Microscopically a section across the long axis of a groove shows a depression of the surface of the cartilage with relatively slight bulging or elevation of the lateral margins. The membranous surface layer may be swollen in the depth of the groove. The cartilage cells are somewhat disturbed in arrangement and more separated in the matrix beneath the marginal bulging. The cartilage beneath shows more or less fibrillation, the long axes of the fibers being at right angles to the joint surface, the fibrillated material extending a varying distance toward the cortical bone (Figs. 6 and 7). Deeper grooves show correspondingly greater changes

both in the character and in the degree of the cell arrangement. Groups of nuclei in a single capsular space are frequent and fibrillation is more pronounced. Occasionally splits or clefts are formed along the fibrils, extending varying distances into the cartilage (Fig. 8). These may reach the cortical bone and sometimes penetrate into it along the vascular tissue projections which extend from the bone into or through the calcified matrix. When this occurs there is a necrosis of the subchondral plate and a production of fibrous tissue in the spaces between the bone trabeculae. This may go on to the formation of cartilage and osteoid tissue, while lateral to such clefts bone production, beginning as an increase in thickness of the calcified matrix, may take place and extend into the cartilage. In this manner a bony thickening of the cortex may occur.

These clefts and accompanying or subsequent changes are much more frequent following other types of degenerative change described below. In our material lesions were found in the subchondral bone only when cartilage changes had reached the bony cortex. We are therefore unable to confirm Hare's hypothesis that focal necrosis in the bone is the primary change. In our specimens the bone changes have been obviously secondary to those of the cartilage.

"Blister" formation is somewhat more common than grooving and may be seen in any joint. Equine joints have furnished the best examples. The first changes noted grossly are small smooth elevations of the surface of the cartilage without color change. These become somewhat flattened at the top, the margins more sharply defined, and the color paler than the surrounding tissue. Rupture usually occurs and the peripheral layers become shreds or fringes projecting above the surface. It appears that coalescence of these blisters forms fringe areas of varying size (Figs. 15 and 16).

Microscopically the early stages in formation are best studied in frozen sections of the joint cartilage. Serial sections of an early elevation show in the center an irregular space bounded by fibrillated cartilage surrounded by a zone, more or less fibrillar, with cells more widely separated than normal (Fig. 12). This change gradually decreases to blend finally with the normal cartilage beyond the swollen areas. Study of a number of these early lesions,

which are located in the peripheral third or half of the cartilage, suggests that the primary change is a focal edema, followed by fibrillation and "blister" formation (Fig. 11). Rupture of such blisters gives rise to fringes. These consist of strands of fibrillated cartilage projecting above the surface, the longer ones being attached at the periphery of the blister, the shorter extending from the base of what now may be termed a small "ulcer" or erosion.

Occasionally on the same joint surface on which the discrete blister formations are seen, shallow pits occur which have slightly raised edges and a smooth surface only slightly paler than the unchanged cartilage. Microscopically these are covered by partly hyalinized fibrocartilage continuous with the upper layers of the normal cartilage (Fig. 13). Beneath the surface layers, and extending a varying distance toward the cortical bone, the cartilage is more or less fibrillated and the nuclear arrangement is disturbed. Such pits appear to result from blister formation followed by loss of the fluid but without fringes being formed, as is the case in those that have ruptured.

Considerable areas of this "fringed" cartilage are found which, as stated above, may have resulted from the coalescence or extension of the blister areas, but may also have originated otherwise, though other mechanisms or sequences of events have not been evident in our material. Traumatism of the apposed surface by the frayed tissue may well account for the fringed area on that side without the preliminary blister formation. Figure 14 shows changes in the cartilage of the proximal end of the humerus of a mule apposed to an eroded, fringed glenoid fossa. The small pale areas are fibrillated, almost myxomatous tissue. In some areas there were some membranous fringes and the entire cartilage showed fibrillation and abnormal arrangement of the cartilage cells.

Clefts extending toward or into the cortical bone occur, as in the case of the grooves. These are often close together along the surface and extend but a short distance into the cartilage, while others, more widely separated, extend deeper, forming irregular masses of cartilage attached at the base to the calcified matrix. Beneath, and in the fringes, the alignment of the cells is markedly disturbed and cell nests are often abundant. Practically always both apposed surfaces show changes though these are usually dif-

ferent in extent, sometimes in type, or both. Grossly the uniformly fringed areas are described as erosions. Relatively low magnification ($\times 10$), however, shows the essentially villous character of the bases which is confirmed by microscopic sections.

The further progress of the erosive degenerative process is characterized by a necrosis and loss of substance of the surface of the ulcer so that the remaining tissue is thinner than the surrounding cartilage. As this loss of substance continues the lesions appear as irregular, discrete and confluent areas eroded to varying depths below the surface of the normal cartilage (Fig. 19). The bases lose the villous character and become dingy gray in color. Such erosions or shallow ulcers were by far the most frequent type of lesion found by us in equines (Fig. 18). They were present in some joint of practically every animal autopsied for this investigation, all of which were destroyed because of disabling lameness. In man the fringed type was the most frequent (Fig. 15). In some of these the fringes projected above the surface but in many there was loss of substance so the fringed bases were definitely below the normal cartilage level. Most of the human bones, however, came from individuals who had given no history of joint disease.

The bases of the deeper ulcers, as observed in horses and mules, have been covered by a layer of dense fibrous tissue though masses of fibrillated hyaline cartilage may remain projecting from the underlying bone. The bone itself has never been bared. In man several advanced cases have shown areas of bare bone though in each instance portions of the surface were covered by fibrous tissue and fibrous and fibrillated hyaline cartilage (Fig. 17). When bone is bared it may be eburnated, but also may show thinning of the cortical layer and actual absence of bony cortex and calcified matrix. The bases of such areas consist of ends and small bridges of trabeculae between masses of fibrous and hyaline cartilage. In such cases vascular fibrous and osteoid tissue extends varying distances into the cortex, replacing the fat marrow between trabeculae (Fig. 20).

Frozen sections of undecalcified cartilage of affected joints show considerable variation in the amount of visible calcium as indicated by staining reactions. The cells near the calcified matrix showed the most but the distribution was not uniform. In some

areas in and near erosions and fringed areas none of the granular deposit may be seen. Further, in apparently normal cartilage, especially in the later age groups, the distribution was often uneven.

The ulceration and loss of substance usually involves both apposed surfaces which then cease to be in contact. Such areas no longer carry their proportion of the stress of the joint, therefore the remaining cartilage must carry the entire load. There is malocclusion of the joint. In such joints hypertrophic changes occur which appear to be compensatory in character.

The first change appears to be hypertrophy, or swelling of moderate degree, of the remaining cartilage at the margins of the bearing surface and extension of the cartilage over the edge of the joint surface, especially in such joints as the patella-femoral. The new or extended cartilage is fibrillated and the cell arrangement is distorted. Then bone formation starts from the calcified matrix and extends into the cartilage in the same manner as occurs beneath ulcerated areas on the original joint surfaces (Fig. 8). As the bone production continues vessels from the marrow enter the process and eventually the new bone becomes continuous with the old and appears of similar or like structure (Figs. 21-24). It has been suggested (Parker⁶) that the pressure in the maloccluded joint forces the bone outward to form these lips or shelves. Except that the pressure must have an influence in the direction the new bone formation will take in relation to the old bone, it does not appear from our material that this is so. That it is compensatory is indicated by the occurrence of the same process directed against the pressure of the joint as seen in Figures 23 and 24, where it seems that the bone is produced in an effort to compensate for the loss of cartilage substance.

Masses of these shelves composed of cartilage, with or without some bone formation, may fail to be firmly united to the bone. In such instances they become pedunculated and later may be separated and free in the joint as "joint mice" (Fig. 21). Nutrition of these free masses appears to be fairly well maintained though in the larger ones the central ossified portions may become necrotic. This maintenance of structure indicates that the nutrition of joint cartilage, to a great extent at least, is from the synovial fluid.

Hypertrophic lesions were neither numerous nor severe in the

horses and mules of our series. These animals are destroyed before the occurrence of such advanced changes as are found in man. Masses of cartilage in the capsule and at the joint margin, however, were occasionally found.

The semilunar cartilages of the knee show degenerative changes more or less in proportion to the degree of change in the cartilage of the apposed femur. These menisci are formed of bundles of fibers embedded in a hyaline matrix, the long axis of the fibers being roughly parallel to the contour of the meniscus. The cross section is triangular, the apex toward the center of the joint area, the base at the periphery. The base is attached by fibrous tissue to the margin of the tibial condyles and the fibrillated ends are attached to the central portion of the bone between the condyles. The medial meniscus is longer and smaller in cross section than the lateral one. The changes in degenerative arthritis are: first, fringes extending onto the central portions from the apices of the menisci; then fibrillation or rather loss of the hyaline matrix so that the cartilages appear as masses of fibers, which may rupture so that the cartilage appears fractured; and finally considerable portions may disappear (Figs. 25 and 26). These changes are usually more advanced in the larger medial meniscus and occasionally in man may be advanced with only relatively minor degeneration of the cartilages of the femur. As observed in our material, they were seldom of advanced degree in equines. In joints in which the menisci show advanced degeneration with loss of substance, new cartilage and bone formation may occur from the intercondylar eminence at or near the attachment of the lateral meniscus. Such formation would appear to be compensatory in that it would tend to limit the lateral motion of the femur on the tibia which the degenerated menisci no longer serve to prevent.

Changes in the synovial membrane lining the joint cavities have not been marked except in a few of the advanced hypertrophic cases in man. Vascular papillary projections from the synovial membrane are practically constant. These are of small size in most mildly affected joints, longer and more abundant in those showing hypertrophic changes. Occasionally in both man and equines there are relatively large (1-2 cm. diameter) pedunculated cystic masses covered by synovial membrane. Occasionally,

attached to and apparently arising from the capsule at its attachment to the bone, pedunculated and sessile masses of cartilage are found evidently arising from this membrane. Rarely such a cartilaginous mass is embedded beneath the synovial membrane in the inner tissues of the capsule.

In advanced hypertrophic cases the synovial fluid may be dark in color and gelatinous in consistence. When this condition has been found in equines there has usually been some evidence of recent injury complicating the disease process.

DISCUSSION

Grooves and blisters, especially the latter, appear from our experience to be the primary and earliest lesions in degenerative arthritis. Grooves are best seen in equines. This type of lesion is easily overlooked in human joints. Practically all writers have described the fissures, clefts and fringe formation, and the extension of the clefts into the subchondral bone. The relation of these to blood vessels has been noted. We have not seen evidence of fracture of the bony plate as noted by Parker but fractures might well be caused by "joint mice" caught between the joint surfaces. As a result of the study of serial sections, these openings from cartilage to marrow appear to be the result primarily of a cartilage fissure extending through the calcified matrix along vessels which appear normally to extend to the cartilage from the marrow beneath. The clefts are localized; that is, the extension into the bone rarely exceeds 1 mm. in the plane at right angles to that of the section. Eight or ten sections at 25 μ often show both termini of these lesions.

Pressure may have something to do with the process in the early stage but the condition may continue after pressure has been removed by loss of substance from both apposed surfaces. Moreover, when new bone is formed above the previous normal level of the bony plate evidence may remain beneath the new bone cortex in the form of fibrous, cartilaginous or osteoid tissue which has not been replaced by fatty marrow. It appears that when the process is complete, as shown in Figures 23 and 24, a normal marrow eventuates as in primary bone formation. We are simply viewing various stages in the breakdown and repair of the joint structure. Therefore, it appears reasonable that abnormal tissues, remnants

of the repair, should be found beneath the joint surface after the process in the immediate area reaches a stage which, though imperfect as compared to the normal, is practically complete or stationary. It is necessary to view more than one plane in studying a three dimensional process if erroneous conclusions are to be avoided.

Several writers have mentioned the increased density of the bone beneath the degenerated areas, usually referring to it as compression of the trabeculae. Some also speak of rarefaction or osteoporosis as a feature of the process in the subchondral bone. As mentioned above, there is marked variation in the character of the subchondral bone. We have been unable to demonstrate in the subchondral bone either increases in density or porosity which could be considered a part of the disease. Certainly these are not early changes. Neither has vascular change nor the necrosis in the bone as described by Hare been evident in our material prior to advanced degeneration of the overlying cartilage.

Increase in density of subchondral bone occurs in areas where the physical nature of the joint indicates that the greater stresses occur. It is possible that due to this density the nutrition of the lower portion of the articular cartilage, which is believed to receive its nutrition from the underlying bone, receives less as a result of such density and relative avascularity and thus is rendered more susceptible to degenerative changes. However, the peripheral portions of the cartilage receive nutrition from the synovial fluid, as is indicated by the condition of the cartilage in "joint mice." From our material it appears that the primary degenerative change starts in the peripheral half or third of the cartilage and this is supported by the studies of others. It thus seems more logical to assume a change in the character of the synovial fluid, rather than in the subchondral bone, as responsible for the primary degenerative process.

Eburnation of the bared bony cortex occurs in advanced lesions in man but such eburnation appears to be the result of new compensatory bone formation which commences in the calcified matrix and extends into the cartilage. Such bone, kept bare by the frictional trauma of an apposed bare bone, may show a dense cortex, often interrupted by areas of imperfect bony closure of the bony plate as seen in Figure 20.

The productive (hypertrophic) changes are similar in man and equines but have been seen in advanced stages only in man. Their compensatory character has been indicated. There has been some argument as to the origin of the new formation of bone and cartilage but in the specimens available to us there seems to be but one source and that is the calcified matrix and subchondral bone. Even in advanced lesions we have not seen evidences of periosteal bone formation or evidence that the connective tissues at the margin of the joint enter into the productive process. Even at the margins where the lips or shelves are formed there is little evidence of new cartilage formation. Instead, there is evidence of proliferation of the cartilage cells near the calcified matrix while the peripheral hyaline cartilage appears edematous or swollen rather than proliferating. In some instances fibrous tissue from the region of the joint margin and capsular attachment extends over a lip or shelf of bone. This fibrous tissue seems to be proliferating and there is sometimes a suggestion of the formation of fibrocartilage but such pictures are unusual in degenerative arthritis and lead to a suspicion that some other factor is present. This construction is the common one in inflammatory arthritis, including the so-called rheumatoid variety in which the fibrous tissue mass extends as a pannus onto the surface of the joint cartilage; the cartilage degenerates, eventually may disappear and fibrous and finally bony ankylosis eventuate.

We have not examined phalangeal joints in which Nichols and Richardson described periosteal bone formation. In these joints the periarticular cartilage masses known as Heberden's nodes are large, as compared to the joint, and it would be difficult to decide their origin because their peripheries blend with the periosteal structures along the shafts of the bones. Certainly in the weight-bearing joints of our series, of all grades of severity, especially in the human specimens, we have not been able to determine periosteal bone formation. In equines this occurs in spavin, for instance, which is primarily not a disease of the joint cartilages, but there is bone production by the periosteum in the region of the capsular attachment which may extend over the joint area and interfere with motion. Spavin may be concurrent with but does not appear to be a part of degenerative arthritis.

The number of individuals, human and equine, that have been

examined routinely at autopsy for joint disease is still too small to determine the incidence of degenerative changes in the joints. Fifty-four Army horses and mules over 10 years old, destroyed because of lameness, had many lesions each in various joints but the extent and severity of the lesions did not always correspond with the clinical findings. That is, there were instances in which the most advanced or extensive lesions were in joints that had not been considered affected, and minor lesions in many joints other than those responsible for the symptoms. No lesions were found in a 6 year old pony that died of encephalitis.

These findings in equines are in accord with those in man. Only 5 of our 7 advanced cases with lipping and other hypertrophic changes had complained of their joints. Two of 18 cases with rather extensive erosions but no definite hypertrophic change had vague symptoms. Forty of the 48 persons whose knee joints were found affected did not complain of joint trouble. Twenty-two of the 40 had mild lesions, 16 had moderately extensive erosions and 2 had advanced lesions with hypertrophic changes on both patella and femur.

The human series of Keefer, Parker, *et al.*,⁸ consisted of knee joints from 100 persons, dead of various diseases. In the combined groups (Keefer's and ours), no lesions were found in 7 in the first two decades of life. Lesions were present in 1 of 2 in the third decade, 5 of 7 in the fourth, 15 of 16 in the fifth, 36 of 37 in the sixth, all of 42 in the seventh, 26 of 27 in the eighth and 15 of 16 in the ninth decade. The findings in even this small number indicate that lesions of degenerative arthritis may occur at any time after the bone and joint structures are completely formed and that after the fifth decade is reached few persons are free from these changes. It will require the examination of a number of different joints of several thousand persons in general autopsy services to determine the incidence of this disease, especially because so few of those affected have clinical symptoms. Roentgen examinations are of great value in determining the presence of relatively early lesions in such joints as those of the vertebrae and fingers. They show ulcers on the condyles of the femur when they are deep enough and can be X-rayed in profile but do not show lesions of the patella and patellar fossae until the hypertrophic changes of the advanced process are present.

Clinical symptoms are of some value. In the inflammatory conditions, including the so-called rheumatoid arthritis, pain, tenderness and some fever are present early and often usher in the process, while in pure degenerative arthritis the process is usually advanced and secondary hypertrophic changes have occurred before symptoms are sufficiently severe to send the patient to a physician.

The seven advanced lesions in our series were well distributed in the age groups. The youngest was 48, the oldest 87. Both of these and two in the eighth decade, age 73 and 76, had syphilis. All seven had arterial disease and three had gangrene of toes. In our subclinical group as a whole, although the numbers are small, the extent of the area involved appeared to increase with advancing age, but exceptions were present. Work done so far suggests that starting at about age 20, the incidence rises with increasing rapidity for the next 20 years. Thereafter the changes are found in practically all. Increase in the extent of the areas involved appears to increase with age but increase in severity — or perhaps better stated as rapidity of progression of the lesions — seems to be due to other factors than age. One is reminded of the great variations in the extent and rapidity of progress of the arterial degeneration in atheromatous arteriosclerosis.

There is little evidence as to the cause or causes of the primary degenerative change. The age incidence suggests that metabolic or rather chemical or physico-chemical changes in the cartilage render it more susceptible to damage by normal wear and tear. The early stages of blister formation bear out this hypothesis. The subchondral bone cortex and the calcified matrix are formed at the limit of the vessel loops from the bone. The formation of this limiting line is gradual. In early childhood the end of the bone is largely cartilaginous and no obvious dividing line can be made out between that portion of the cartilage which will remain as such and that which will eventually become bone. The completion of the bone cortex and the superimposed calcified matrix may well mark a quantitative change in the nutrition brought to the joint cartilage. There are some data on this point, though how such chemical and chemico-physical changes as have been found are related to degeneration of the cartilage is unknown.

Hoffmann, Lehmann and Wertheimer are quoted by Bywaters ¹⁴

as finding that the glycogen content of cartilage varies with the kind of cartilage and the age of the animal, also in different individuals, variations being between 0.02 and 0.6 per cent. It was also indicated that there is a diminution with age and with increasing calcification of the cartilages.

The calcium content of cartilage can scarcely be determined with sufficient accuracy for comparative studies because of the impossibility of obtaining uniform specimens for analysis. Unless samples include the base as well as the periphery of the cartilage without the inclusion of underlying calcified matrix and bone, and this is practically impossible, comparative analyses would be of little value. Our studies of frozen sections indicate that there is a considerable variation in the amount of visible calcium between the deep and superficial portions of the cartilage and between the cartilage of children and adults. Further, there is much variation in the evenness of its distribution in the cartilages of the later age groups. Whether this variation is quantitative chemically or whether it is one of the physical-chemical state of the calcium, we do not know. The degenerative changes, especially the splitting, suggest a loss of elasticity. Perhaps analogous conditions are the increase in calcium found accompanying loss of elasticity in the aorta and lens of the eye. Disturbance of chemical equilibrium might cause a change of the calcium from a colloid to an insoluble state, while simple dehydration, causing a relative increase in the calcium content, might be responsible for lack of cartilage elasticity. Certainly degenerated cartilages appear dry as compared with the normal, but comparative analyses for water content would be as difficult as those for calcium. Further, the cause of dehydration, if existent, might well be related to changes in osmotic pressure caused by decrease in salt content or change in the chemical-physical state.

Believing that a dietary deficiency with mineral imbalance was the cause of the lameness in the group from which our equine cases were taken, their diet was modified by adding alfalfa hay, which corrected the calcium deficiency, balanced the $\text{CaO-P}_2\text{O}_5$ ratio and added vitamins, especially vitamins A and D. A number of our cases had been on this improved diet for 2 years but we were unable to make out any evidence of a repair process other than the compensatory hypertrophic changes described. The period was

not sufficiently long to determine the effect of the new diet on the incidence of clinical lameness.

CONCLUSIONS

1. Degenerative arthritis is a definite disease entity of unknown etiology which commences as a degeneration of joint cartilage and involves bone only secondarily.

2. It exists in many animal species and is an important cause of disability in man and equines.

3. The lesions in man and horses and mules are practically identical in character, though the most advanced lesions are not ordinarily found in equines because the resulting disability causes them to be destroyed before the changes reach such a stage.

4. Though a relatively few examinations have been made, the lesions of degenerative arthritis have not been seen in man prior to the third decade, but after this their incidence increases rapidly so that they are almost universal when the fifth decade is reached.

5. A relatively small proportion of the lesions give rise to symptoms but this proportion increases with advancing age as does the extent of the areas involved.

6. Involvement of the cartilage alone often appears to be symptomless. When pain occurs it probably usually results from pressure on subchondral bone which has either been denuded of cartilage or more or less damaged by extension of the degenerative change. In those cases in which loose pieces of cartilage or bone (joint mice) are present in the joint, pain may be produced as a result of their getting between the joint surfaces.

7. Loss of cartilage substance, causing malocclusion of the joint, is followed by bone production from the calcified matrix and subchondral bone. This appears to be a compensatory effort to replace the degenerated cartilage with bone and, though secondary, has given the name hypertrophic arthritis to the disease. This new bone formation is rare in man except in the clinical disease in which it is usually present.

8. Symptomatic degenerative arthritis occurs in equines from less advanced lesions than in man. It appears that the greater the physical activity of the individual, man or animal, the more serious does this condition become.

9. While the area of cartilage, the number of joints involved,

and the incidence of clinical arthritis increase with advancing age, rapid progress of the disease to severe disability appears to be due to other factors as it may occur in the fifth decade or earlier, as well as in later life.

10. Ankylosis, fibrous or bony, does not occur in this condition though compensatory bone formation may be responsible for limitation of joint motion.

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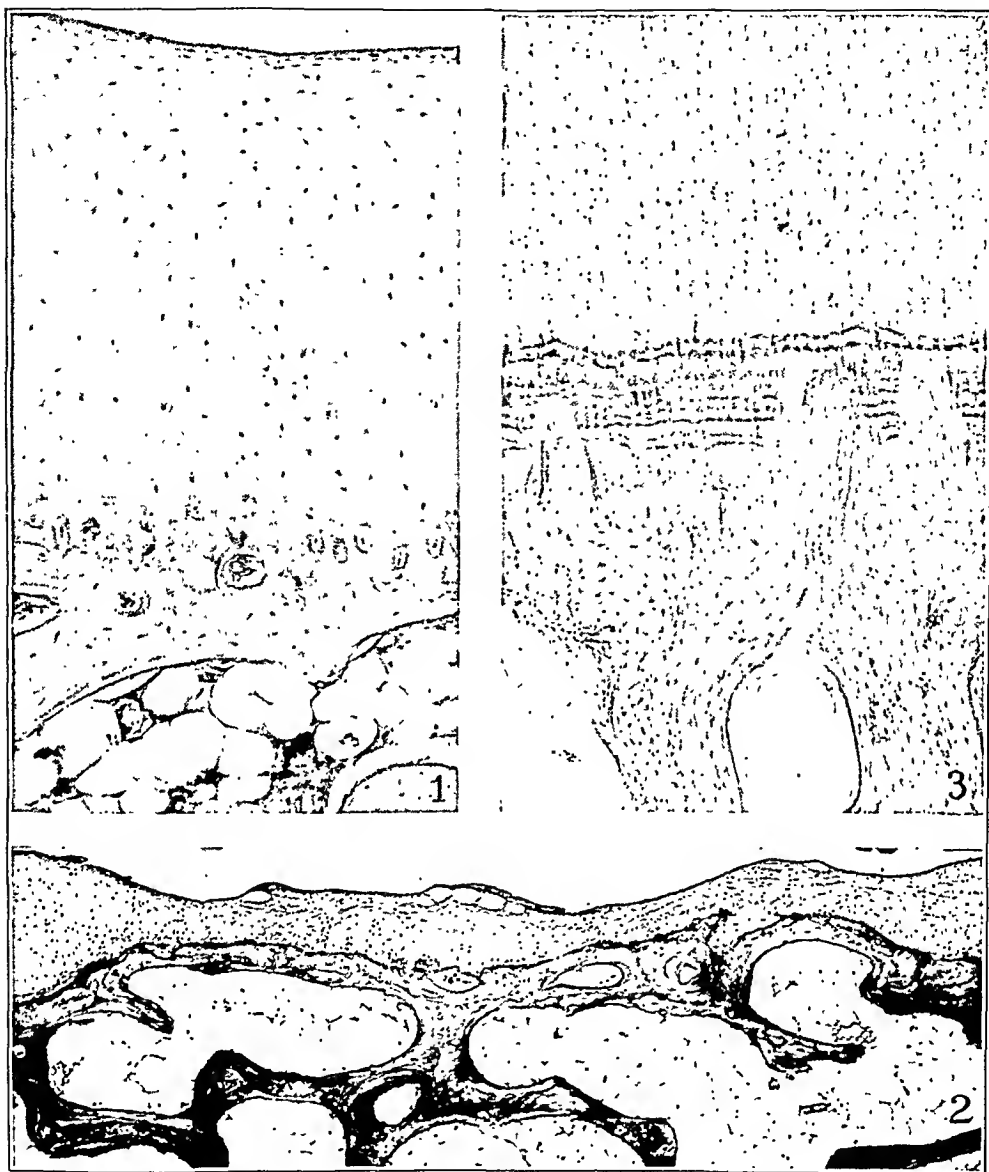
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DESCRIPTION OF PLATES

PLATE 48

- FIG. 1. Normal human joint cartilage from condyle of femur. Note irregular distribution of cartilage cells.
- FIG. 2. Ulcer on femoral condyle. Base covered by synovial membrane which shows cyst formation. Calcified matrix thin with bony spur extending into cartilage to right of center.
- FIG. 3. Normal equine lower cartilage, calcified matrix and cortex of subchondral bone. Note vessels penetrating nearly through calcified matrix which shows striae of calcification (*c.f.* Fig. 1 in which calcified matrix is not striated). Both types are found in man and equines. Note regular arrangement of the cartilage cells.



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PLATE 49

- FIG. 4. Shallow grooves and "blister" in human patella.
- FIG. 5. Shallow and deep grooves in the cartilage of the distal end of the large metacarpal bone of a horse. The darkest streaks showed deep red at the base.
- FIG. 6. Shallow grooves in human joint cartilage cut across their long axis. Note soft tissue in base of the one at the left.
- FIG. 7. Shallow groove in equine joint cartilage.
- FIG. 8. Deep groove in equine joint cartilage. A cleft extends from base of groove into cortex of bone. Vascular fibrous tissue replaces bone about the cleft and extends into marrow spaces at lower right. Beginning bone formation replaces calcified matrix and extends into cartilage. Nuclear arrangement of cartilage is disturbed and there is necrosis of the fringed surface on the right margin of the groove.



PLATE 50

FIG. 9. Large "blister" on a human patella.

FIG. 10. "Blisters" and "pits" in cartilage of an equine patella.

FIG. 11. Mound of edematous cartilage on left and ruptured "blister" on right. Frozen section from equine patella cartilage which grossly was similar to specimen shown in Fig. 10.

FIG. 12. Early "blister" formation. Frozen section of equine patella cartilage.

FIG. 13. "Pit" in equine patella cartilage from specimen similar to that seen in Fig. 10.

FIG. 14. Membranous fringes on the surface and multiple areas of pale, almost myxomatous tissue in cartilage of equine humerus which was apposed to a fringed glenoid fossa.

FIG. 15. Fringed eroded patella fossa, upper left, and deep ulcer on condyle, lower left, of human femur. Patient had not complained of joint symptoms.

FIG. 16. Fringe areas and a few small "blisters" on femoral condyles of a mule. Animal had been affected with shifting lameness and many joints showed degenerative arthritis.

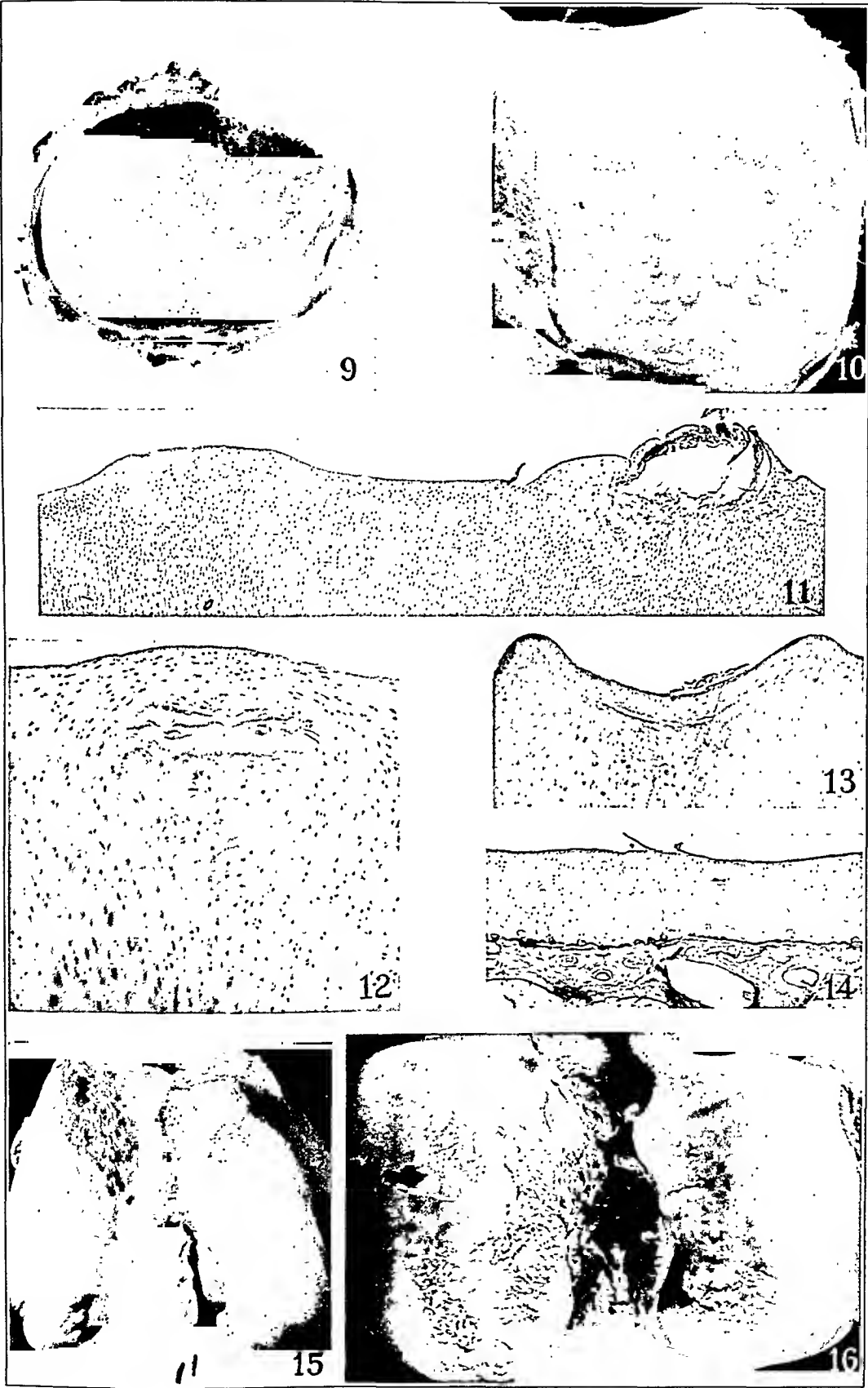


PLATE 51

- FIG. 17. Advanced degenerative arthritis with hypertrophic changes (see Fig. 21 for lateral view). Lower end of human femur. Condyles are partly denuded but there is little eburnation. Note island of swollen cartilage in the ulcerated area on the right.
- FIG. 18. Erosions in the cartilage of the patella fossa and femoral condyles of a mule destroyed for lameness.
- FIG. 19. Ulcer in human cartilage showing marked fibrillation fissures or clefts and bone formation from region of the calcified matrix. Note that there is no change in the density of the subchondral bone.
- FIG. 20. Section from human joint which showed ulceration similar to that seen in Fig. 17. Ulcer surface composed of ends of trabeculae and masses of fibrillated cartilage. Marrow spaces near surface are filled with fibrous and osteoid tissue. There is lipping at the margin from new bone growth which is covered with swollen fibrillated cartilage.

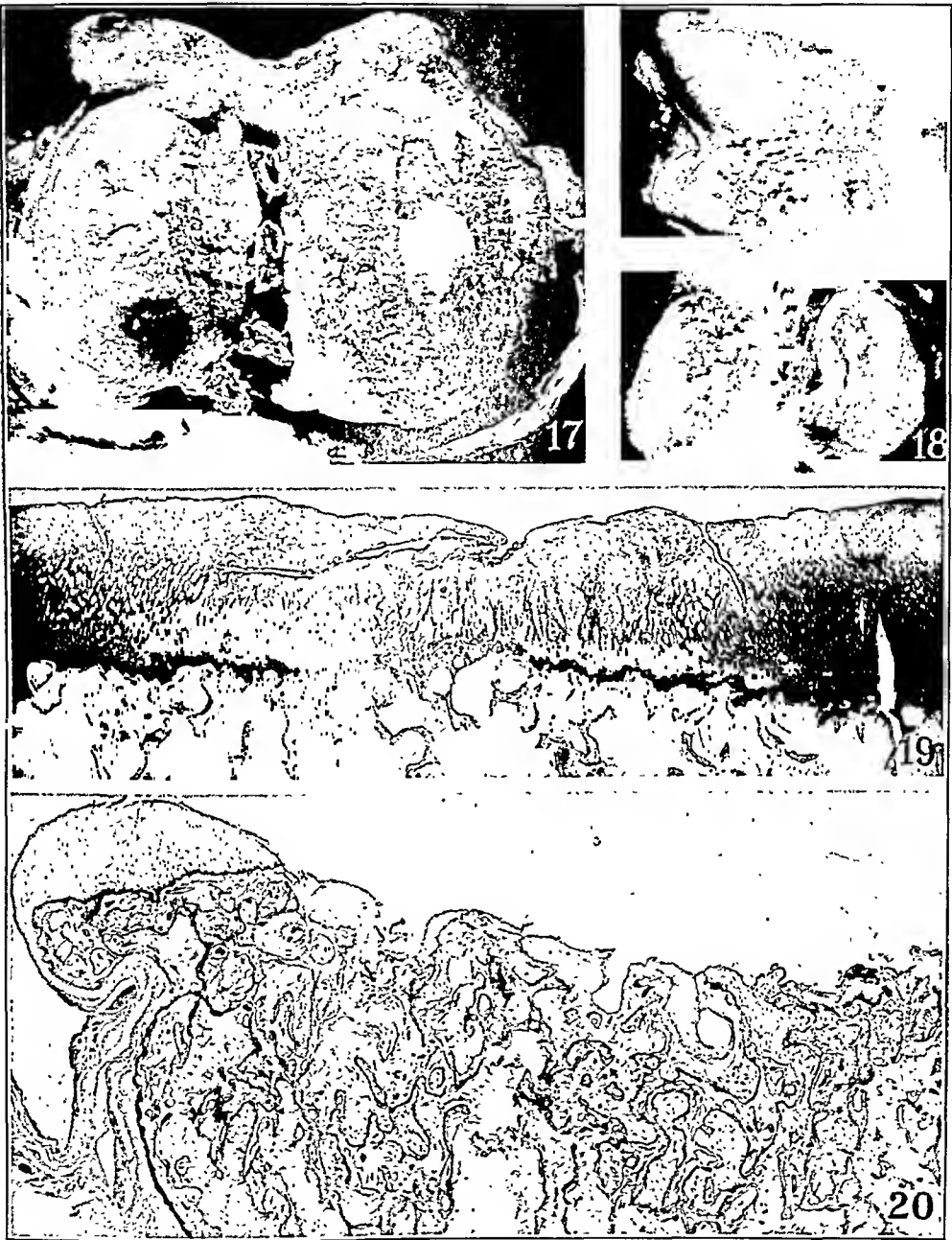


PLATE 52

- FIG. 21. Advanced lipping of patella fossa and condyle of human femur (see Fig. 17).
- FIG. 22. Shelf formation on equine patella. Note pedunculated mass arising from bone margin. Fringed erosion occupies upper half of joint surface.
- FIG. 23. Section of lipped margin of human patella fossa which grossly was less advanced than that shown in Fig. 21. New bone, which appears normal, rises above the old cortex and is covered by thin fibrillated cartilage. There was extensive deep ulceration of patella and patella fossa. Note extension on to old cortex.
- FIG. 24. Section from the opposite side of bone of Fig. 22, showing cross section of the lip or shelf. Note similarity of Figs. 23 and 24.
- FIG. 25. Human tibia. Mesial semilunar cartilage (left) shows fibrillation for a short distance from its attachment and a small fringe. Lateral meniscus essentially normal, early "blister" formation in cartilage on right.
- FIG. 26. Human tibia. Lateral meniscus shows rather large pedunculated masses extended onto the eroded joint cartilage. Medial meniscus largely destroyed. Mass of bone covered with cartilage at lower center which extended to cervical ligament.





STUDIES ON EXPERIMENTAL RICKETS IN RATS *

II. THE HEALING PROCESS IN THE HEAD OF THE TIBIA AND OTHER BONES

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INTRODUCTION

During the decade and a half in which serious experimental work on rickets has been carried on by the use of rats and other animals, remarkably little attention has been given to the study of the pathological details in the bones, except so much as has been necessary for diagnostic purposes. In an earlier paper (1934) we presented an account of the outstanding structural changes observed in bones during the active phase of rickets, with special reference to the behavior of the epiphyseal cartilage in the head of the tibia. In this paper we present a comprehensive account of the healing process.

The best account we have seen of the healing process in experimental animals is that by Pappenheimer (1922) on the healing of the ribs of rats under the influence of cod liver oil. Our findings are in complete keeping with his, but we have carried the study to greater detail than he did. From a study of numerous published pictures of bones of rats showing healing of rickets, mostly at low magnifications, we conclude that there is nothing unusual in our material. We present our account with confidence in its wide applicability.

MATERIAL AND METHODS

Number of Rats and Diets Employed

The observations recorded in the present paper are based on the study of 125 albino rats as follows: (1) normal controls fed a balanced diet, 26 rats; (2) various stages of active rickets, 31 rats; and (3) rickets in various healing stages, 68 rats.

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In each instance the rachitic condition was produced by the Steenbock-Black rachitogenic diet with high calcium, low phosphorus and deficient vitamin D.

Healing in the 68 rats was induced as follows: (1) by irradiated ergosterol (Viosterol), 0.00001 mg. daily for 5 days, 39 rats; (2) cod liver oil, 2 drops 3 times a week during experiment, 16 rats; (3) return to balanced diet, 3 rats; and (4) spontaneous healing, 10 rats. In all four groups the course of healing was the same, though there were differences in rate and permanence.

The young rats were fed the rachitogenic diet at the age of 4 weeks. Rickets was usually well developed by the age of 8 weeks. At the age of 8 to 9 weeks the feeding of the curative agent was begun. A few rats were carried as far as the age of 13 weeks, 5 weeks after the beginning of the curative regimen. The material includes rats under experimental handling at all seasons of the year beginning with the autumn of 1931 and ending with the spring of 1935.

Methods of Study

X-ray photographs were taken of all rats under light ether anesthesia at the time of sacrifice, and of most of those in the healing series at intervals of a week or less.

Bones were fixed in Bouin's fluid, usually at body temperature. Decalcification was in Müller's fluid (in which the potassium dichromate is the essential ingredient). Bones thus decalcified give a strong staining differentiation between calcified and non-calcified areas, which is lacking with other decalcifying agents. The stains used were Delafield's, Ehrlich's, or Harris' hematoxylin, usually followed by eosin. From the head of the tibia and the distal end of the femur of 35 rats we made a silver nitrate preparation as in the commonly used "line test," a process that blackens deposits of calcium salts. For these 35 rats we have thus three records: (1) X-ray photographs at weekly or more frequent intervals; (2) stained microscopic sections; and (3) silver preparations. For all others we have two records: X-ray photographs and microscopic sections. We studied X-ray films of all the bones of the legs of all rats, and microscopic sections from the distal end of the femur and of the radius and ulna, but our main study was on the head of the tibia. This bone was selected because of its

good size, rapid growth and general suitability, and because it is one of the bones of the leg which carries the weight of the animal. Moreover, there is special need for knowledge of its structure, because the head of the tibia has come more and more to be used by students of rickets in measuring the progress of healing.

The Identification of Calcified Areas

Each of the three methods we have employed gives useful information concerning the extent of calcification.

X-Ray Photographs: X-ray photographs portray a shadow of the total mineral salts, mostly of calcium, but with very little structural detail in bones as small as those of rats. Nor can X-ray photographs be advantageously studied under magnifications of more than a very few diameters (Fig. 1).

Silver Nitrate Preparations: The silver preparations show calcified areas with some detail, and may with profit be studied and photographed under magnifications of several diameters. Figures 5 to 10 show photographs of bones prepared in this way, the surface shown being made by splitting the head of the bone longitudinally.

Hematoxylin and Eosin Stain: It has been customary for a good many years to interpret a certain deep hematoxylin stain as indicative of calcification in bone and cartilage, even though the tissue has been subjected to decalcification before staining. There are some, however, who question whether such staining is indicative of calcification. The value of much of the work on both healthy and diseased bones depends on the reliability of this reaction. We have made the following observations of differential staining, which we have come definitely to believe identifies calcified areas, the certainty in our minds growing from the constant agreement between the pictures presented by X-ray photographs, silver preparations, and hematoxylin-eosin sections.

Differential Staining in the Epiphyseal Cartilage: In the epiphyseal cartilage of normal bones, the part of the matrix that is generally considered to be without calcification has a light gray color, or at most a moderate purple cast. In the portions of the matrix considered to be calcified there is a very deep purple hematoxylin stain with no trace of eosin coloring (Fig. 21). The cartilage remnants in the interior of recent normal bone trabeculae

show the same deep stain (Fig. 23). In severe rickets there is none of this coloring in the epiphyseal cartilage but it is seen in the cartilage remnants in the interior of the bone trabeculae that were formed before the onset of rickets.

Differential Staining in Osseous Structures: In both normal and rachitic bones the osteoid areas stain a uniform red color by eosin with little or no trace of hematoxylin. When hematoxylin alone is used, such areas are pale gray (Figs. 2 and 3). In areas commonly believed to be calcified, both hematoxylin and eosin participate, producing a purple color with more red than in calcified cartilage. Over the greater part of such areas the coloring is uniform, showing no granular structure, but near the surface of rapidly growing normal trabeculae, and in rachitic bones, the coloring may be in the form of purple granules (Figs. 2 and 3).

Severity of Rickets at Beginning of Healing

In the present paper we describe the healing of only the severe form of rickets, such as used by numerous workers for the assay of antirachitic substances. We considered rickets to be severe enough for our purposes when X-ray photographs showed the uncalcified epiphyseal cartilage in the head of the tibia to have attained a thickness of 1.5 to 2 mm. (Fig. 1). This condition was usually attained at the age of 8 to 9 weeks. At the same time growth had become decidedly subnormal and the rats had fallen well below the normal weight for rats of their age.

THE HEALING PROCESS IN THE HEAD OF THE TIBIA

Scales for Measuring the Progress of Healing

The "Line Test": The "line test" of McCollum *et al.* (1922) has been widely used in estimating the rate of healing. The four stages in common use are well illustrated in the colored figures of Bills and McDonald (1926). Figures 5 to 10 show the progress of calcification by this method.

X-Ray Scales: The use of the X-ray has also gained some vogue and has many advantages, though also some limitations. An elaborate X-ray scale has been devised by Bourdillon *et al.* (1931). It includes twelve stages which the authors have illustrated by an excellent series of reproductions. We have adopted it as that

against which we measure our microscopic and silver preparations. We have 639 X-ray photographs of the 125 rats in our series (as many as 17 of 1 rat), which we have grouped according to the twelve stages. We do not know whether so elaborate a scale as this is advantageous in the average bio-assay work, but we have found it exceedingly illuminating in our analysis of the healing process.

Our Interpretation of Bourdillon's Scale: We found it necessary to select a series of X-ray photographs from our own material to use as a standard, because Bourdillon used a straight anteroposterior view of the knee joint with the leg extended while we had been photographing our rats as the animal lay ventral side down under light ether anesthesia with the knee somewhat flexed. This position gave a more lateral view of the head of the tibia. We have made every effort to retain Bourdillon's intervals.

The Progress of Healing as Seen in X-Ray Photographs (Fig. 1)

Severe Rickets: No trace of calcification in cartilage region.

Stage 1: Faint diffuse shadow of new calcium deposit about midway between epiphyseal bone and end of shaft. Does not extend to posterior surface of bone. This new calcium deposit spreads toward the end of the shaft, as described in the following stages.

Stage 2: Shadow more dense, and at anterior margin of bone has come to form a narrow definite line.

Stage 3: Line shadow broader and extending half-way to posterior margin of bone. Diffuse shadow nearly to posterior margin.

Stage 4: Definite strong shadow extending from anterior to posterior surface of bone.

Stage 5: Shadow stronger and broader, and approaching end of shaft. Anterior margin of shadow forms sharp outline of surface of bone.

Stage 6: Shadow still broader and beginning to join end of shaft. Surface of bone well defined at both anterior and posterior margins of new calcium deposit.

Stage 7: New calcium, as shown by shadow, joined to end of shaft or very nearly so. Head of bone still has definitely rachitic shape as shown by well developed shoulder at end of shaft.

Stages 8, 9, 10, 11 and 12: In these stages the shape of the head undergoes correction by the gradual obliteration of the shoulder

at the end of the old shaft. During this process the shoulder recedes from the epiphyseal cartilage and at the same time becomes less prominent. At about Stage 9 the epiphyseal cartilage begins to grow thinner and by Stage 11 is about normal, though this is a point not clearly seen unless the plane of the photograph is just right. Stage 12 shows practically normal form and structure.

Parallel Between Line Test and X-Ray Stages: As nearly as we can tell by comparison with the figures of Bills and McDonald (1926) and others, and by study of our own silver preparations, the correspondence between the four line test stages and our twelve X-ray stages is about as shown in Table I.

TABLE I
Comparison of Line Test and X-Ray Stages

Line test stages		X-ray stages
+	equivalent to	1
++	" "	2
+++	" "	5 or 6
++++	" "	about 8

The General Nature of the Healing Process

In the study of healing it is important to keep in mind at all times the two main structural elements involved, *i.e.* the marrow and the solid tissues (bone and cartilage). It must be remembered also that both bone and cartilage have two constituent parts—the cells and the intervening matrix. The matrix in turn has two main parts—the organic portion and the calcium salts. The progress of calcification during healing is shown in the X-ray photographs just described and serves as a background for detailed study of structural changes. The marrow must be recognized as the vehicle through which the cartilage and bone are in large part nourished; by which they are built up and destroyed; and through which calcium salts are supplied and removed.

In our observations, healing began in the metaphysis close to the thin part of the epiphyseal cartilage, whence it quickly spread laterally into the projecting masses of thick cartilage at about the same level. From this beginning the reorganization spread toward the shaft in both the area of the metaphysis and the cartilage masses (Figs. 1, 6, 7 and 8). While this conspicuous progressive

reorganization was going on there was also taking place a quite generally distributed phase of healing.

The healing process may conveniently be described under the following headings: (1) the rachitic metaphysis; (2) the epiphyseal cartilage; (3) the diaphysis (shaft); (4) the epiphyseal center; (5) correction of shape; and (6) the marrow. Changes that are going on at the same time must of necessity be described consecutively.

Though the general structure of rachitic bones is familiar to most readers, it will be necessary to include in the account of healing short notes on certain features of rachitic bones.

The Reorganization of the Rachitic Metaphysis

The Rachitic Metaphysis: The rachitic metaphysis is a characteristic and important feature of rachitic bones which has received very little attention in papers dealing with rickets. It takes the place of the portions of the thickened cartilage removed during rickets (Figs. 5 and 11). It corresponds to the primary spongiosa of normal bones, but lack of calcification causes its structure to be quite different. It consists of cartilage remnants including both cells and matrix, upon which osteoid has been deposited in considerable amounts (Fig. 17). The cartilage-osteoid trabeculae of this region have the form of rather irregular masses, with a tendency to longitudinal orientation. Though these trabeculae in general appearance resemble osteoid rather than cartilage, the uncalcified cartilage matrix persists within them, and is destined to emerge during healing and to dominate the form and orientation of the trabeculae in the restored bone, as will be pointed out later. The marrow spaces, as seen in sections, are about equal in area to the trabeculae. In no case of severe rickets did we observe any calcification in the rachitic metaphysis.

The Cartilage-Metaphysis Junction: An important region in the consideration of healing is the border zone of the metaphysis, where it joins the thinner portions of the epiphyseal cartilage, because it is there that the marrow has made its greatest advance into the cartilage, and it is there that we have observed the earliest healing changes. Throughout the entire border area there were at intervals narrow projecting masses of cartilage which extended into the metaphysis where they became incased with osteoid, thus

making their contribution to the typical structure of the rachitic metaphysis (Fig. 17). Between these projecting cartilage masses were areas of vascular marrow which terminated against the cartilage. There the vascular channels often became considerably expanded, and there were frequently some erythrocytes outside their endothelial walls. In more advanced rickets, when cartilage removal had ceased, the vascular tissue at the cartilage border became largely replaced by cellular connective tissue, while in still more extreme cases the spaces had become largely filled with osteoid tissue (Fig. 17). The superficial region of a bone was likely to represent a more advanced condition than the interior.

The Beginning of Healing as Seen in X-Ray Photographs: In X-ray photographs the first indication of healing was, as generally recognized by students of rachitic bones, a faint shadow appearing in the uncalcified region about midway between the epiphysis and the end of the shaft (Fig. 1). It indicates the general location of the calcification but does not give precise information about the tissue elements involved. The first appearance of this shadow defines Stage 1.

The Beginning of Healing as Seen in Silver Nitrate Preparations: Silver nitrate preparations showed that this earliest calcification (the so-called "line") was in the metaphysis close to the edge of the cartilage (Fig. 6). It had the form of a flattened ring extending inward from the surface of the tibia from which beginning (Stage 1) calcification soon extended inward, until during Stage 2 the ring had become a complete plate (Fig. 7). This early spread of calcification was accomplished by invasion not only of the entire cartilage-metaphysis border zone, but also by extension into the projecting masses of the thick cartilage. While this spread was taking place the zone of calcification also grew thicker by extension into the metaphysis toward the shaft.

A few of our preparations showed a second, rather extensive area of calcification at early stages, deeper in the metaphysis, but this second area was not very common nor did it appear to play an important part in determining the course of healing. Figure 6 shows a bone with this calcified area (Ca 2), while Figure 7 illustrates the more common type which does not have it.

The Beginning of Healing as Seen in Stained Sections: In hematoxylin-eosin sections the first observed changes involved tissue

structure rather than calcification, *i.e.*, enlargement of the marrow spaces in the edge of the metaphysis close to the edge of the cartilage. This enlargement of spaces involved removal of osteoid, where it had clogged the spaces, and a loosening of the cellular structure where there had been dense masses of connective tissue cells. At the same time there took place a reduction in the size of the large vascular channels. The result was the early establishment of a more normal marrow in this region, composed of moderate sized vascular channels surrounded by connective tissue cells (*cf.* Figs. 17, 18 and 22). This early enlargement of the marrow spaces exposed short portions of cartilage trabeculae, connecting on the one hand with the general cartilage mass, and on the other with the cartilage-osteoid trabeculae of the metaphysis.

Earliest Calcification Seen in Stained Sections: We could not detect calcification quite as early in hematoxylin-eosin sections as in X-ray films and silver preparations. It was seen first in the exposed longitudinal cartilage trabeculae, in the edge of the metaphysis bordering the epiphyseal cartilage (Zone 1), and also very faintly in some of the osteoid masses of the adjacent region of the metaphysis (Zone 2). This calcified area corresponds in position to the earliest calcification seen in silver preparations (Fig. 6). In slightly more advanced healing stages (Stages 1 and 2) calcification became increasingly more evident in these regions (Figs. 4 and 18).

Though we have not in hematoxylin-eosin sections observed the very earliest calcification we have reason to believe that it occurs in the masses of osteoid which are very early removed in the first enlargement of marrow spaces close to the edge of the cartilage. This conclusion is supported by the observation that in all later stages the removal of osteoid was preceded by its calcification.

The reorganization of the entire metaphysis was accomplished essentially by a spreading of the processes whose beginnings have just been described.

Progressive Reorganization Processes: The spread of the healing process from the early "line" toward the shaft was carried on thus: calcification of the osteoid began in the metaphysis close to the edge of the cartilage, and progressed steadily toward the shaft, which it reached at about Stage 6. Before the calcified band had become very wide, there began the removal of the calcified osteoid

close to the cartilage. This removal process advanced steadily toward the shaft, always keeping some distance (a somewhat variable distance) behind the calcification front. As the osteoid became removed the exposed cartilage trabeculae became calcified, the calcification of cartilage trabeculae progressing toward the shaft as rapidly as did the removal of osteoid, but a short distance behind.

During the early stages of healing there were thus seen in the metaphysis three zones as follows: (1) adjacent to the epiphyseal cartilage a steadily widening zone characterized by numerous, slender, rather parallel, calcified cartilage trabeculae, with very little osteoid on them (Figs. 4, 7, 12, 18 and 19). (2) There followed a zone in which the masses of osteoid had undergone dilute calcification, but the contained cartilage trabeculae were not calcified. This zone was constantly moving toward the shaft. This zone is shown in silver preparations as irregular masses, in contrast with the slender parallel bars of Zone 1 (Fig. 7). It also shows clearly in stained sections (Figs. 4, 18 and 19). (3) Between the calcified osteoid area and the end of the shaft was an uncalcified zone, which grew continually narrower until it became obliterated when calcification reached the end of the shaft at about Stage 6 (Figs. 7 and 8).

General Reorganization Processes: While the progressive healing changes described above were going on, bringing about the widening of the zone of calcified cartilage trabeculae, there was also going on a less rapid removal of osteoid from the surface of trabeculae in the uncalcified zone. This produced a general reduction in size of trabeculae and enlargement of marrow spaces and caused the distinction between zones gradually to become less sharp, so that by Stage 5 or 6 there was no longer a clear-cut zonation (Figs. 8, 13 and 20).

The general arrangement of the trabeculae during these early stages of healing became increasingly more parallel and regular, the change being due to the removal of the osteoid, so that the parallel cartilage trabeculae, which had been largely obscured during rickets, came to dominate the pattern of the metaphysis as they do in normal bones. This result was made possible because the lacunae in the thickened cartilage had largely maintained their columnar arrangement, even through temporary submergence in

the rachitic metaphysis. Had the cell columns been greatly distorted the attainment of a normal pattern of trabeculae would have been much delayed.

The Union of the New Deposit of Calcium with the End of the Shaft: It will be noted that at Stage 6 (Fig. 1) the X-ray films do not show that the new calcium deposit had united with the end of the shaft, while silver and hematoxylin-eosin sections show that such union had taken place. The answer to the apparent discrepancy seems to lie in the fact that in the metaphysis near the end of the shaft, during Stages 5 and 6 the trabeculae were rather few and the marrow spaces large so that the total calcium at this level was not enough to throw a dense shadow (Figs. 8 and 13).

The later reorganization of the metaphysis is described along with the restoration of the epiphyseal cartilage.

The Reorganization of the Epiphyseal Cartilage

In considering the restoration of the epiphyseal cartilage to normal thickness and structure it is necessary to treat separately its two portions: (1) the thinner part or parts which have been reduced in thickness during the progress of rickets; and (2) the projecting masses which remain of full thickness, still resting upon the end of the shaft (Figs. 5 and 11). For details see our earlier paper (1934).

Removal of the Thick Parts of the Epiphyseal Cartilage: The thick projecting parts of the cartilage were all removed before there was any general reduction of the thinner portions. Removal of these portions was brought about by lateral erosion from the metaphysis, not by frontal attack from the end of the shaft. The removal progressed in such a manner that the last portions to be removed were usually isolated masses near the end of the shaft (Figs. 8 and 13).

The first step in the process of removal was the lateral spread of calcification, beginning at about Stage 2, from the early calcification area into these projecting masses (Figs. 6 and 7). This calcification soon extended clear across, and later spread toward the shaft.

Following its calcification the cartilage was promptly invaded by marrow masses, which usually entered from the side and later proceeded in a more longitudinal direction toward the shaft. This

invading marrow removed most of the uncalcified transverse walls between lacunae in the columns, and also some of the calcified longitudinal walls between columns of cells, leaving the usual, rather parallel pattern of calcified cartilage trabeculae. The fact that the columns of cells in the thickened cartilage were maintained without much distortion made possible this parallel arrangement. Upon these trabeculae, osteoid was promptly deposited in about the amount characteristic of normal bone trabeculae.

The removal of the projecting cartilage masses was largely completed by Stage 6, and entirely so by Stage 7 or 8, at which time the entire cartilage had become reduced to a rather uniform thickness of about double its normal measure (Figs. 13 and 14). With the removal of the projecting cartilage masses, the entire edge of the cartilage and the marrow adjacent to it assumed a rather normal appearance except that calcification extended inward only one or two cells (Fig. 22) instead of the normal extent of three or four (Fig. 21).

General Reduction of the Cartilage in Thickness: At about Stage 9 the general advance of the marrow into the cartilage first began, as shown both by microscopic observations of the removal front, and by measurable reduction in the thickness of the cartilage. At about Stage 10 calcification first extended into the cartilage to about its normal distance of three or four cells. At this time the number of fully enlarged cells in the cartilage columns was still considerably in excess of the normal number. By Stage 11 or 12 the cartilage was reduced to its normal thickness of about $350\ \mu$ (sometimes less) and at the same time the number of hypertrophied cells was reduced to the normal number of three or four (Figs. 15 and 21).

Multiplication of Cartilage Cells: In a previous paper (1934), we recorded our observations that the cells of the epiphyseal cartilage ceased multiplication and the bone ceased to elongate after rickets became severe. We have also observed that during healing the bone did not again begin to elongate until about the close of the healing process, at which time the epiphyseal cartilage had assumed normal structure. We have not made careful search for mitotic figures at the completion of healing, but cell division doubtless was resumed at that time inasmuch as the bone began to elongate.

The Cartilage-Metaphysis Area During the Later Stages of Healing

The trabeculae produced by the reorganization of the thick portions of the cartilage resembled very closely in shape and attitude those formed in the adjacent metaphysis. Both areas contributed to the building of a new end for the shaft, terminating against the restored epiphyseal cartilage.

The later phase (Stages 7 to 12) of the reconstruction of this region involved increasing calcification of the cartilage and osteoid in the trabeculae. At Stage 6 there was very little calcification in the osteoid on these trabeculae, though in this respect there was considerable individual variation (Fig. 22). Beginning with Stage 9 there was usually complete dense calcification, though in some rats the attainment of this condition was delayed until Stage 12. There was also continued, though not radical modification of the form and attitude of the trabeculae, brought about by local removal and addition of osseous material. The result was that the structure in this region grew more and more normal (Figs. 13-16).

The Parallelizing of the Divergent Bone Trabeculae: Throughout the later healing stages there persisted one feature which marked the newly reorganized spongiosa as having come through a rachitic period, *i.e.*, the considerable radial divergence of the trabeculae, a direct result of the characteristic divergence of the cells columns in the rachitic cartilage, in contrast with the parallel arrangement in normal bones (Dodds and Cameron, 1934). The fact that this divergence of the trabeculae emerged in the late healing stages, after long submergence in the uncalcified rachitic metaphysis, is further striking evidence of the dominance of the cartilage pattern in determining the form and attitude the bony trabeculae assume. Figure 14 shows this divergence clearly at Stage 8. Figure 15 illustrates a late stage in the correction of this defect, while Figure 16 shows the parallel arrangement in a normal bone.

The Reorganization of the End of the Shaft

During severe rickets, the end of the shaft was clearly differentiated from the cartilage-metaphysis region because of the persistence of calcification in the pre-rachitic cartilage-bone trabeculae

in the shaft (Figs. 5 and 11). As might be expected, these trabeculae became less robust and more numerous toward the end of the shaft, just as they do in normal bones, but in severe rickets we found them to be much distorted, smaller, and less numerous than in normal bones (*cf.* Figs. 11 and 16). This condition explains the blurring commonly observed in the end of the rachitic shaft in X-ray photographs. Upon these calcified remnants there had been deposited much osteoid, sufficient to reduce greatly the size of the marrow spaces. The end of the shaft in all of our bones had the typical concave or "cupped" form which characterizes rachitic bones.

No special study was given to the cortical bone of the shaft, except to observe that in advanced rickets there was a thick layer of osteoid on both its outer and inner surfaces and that its calcified portion had become somewhat reduced in thickness.

During the first few stages of healing the spongiosa of the shaft with its remnants of pre-rachitic calcification was undergoing reorganization at the same time as the adjacent metaphysis, and there was a general similarity between the changes in the two regions. The osteoid, which had become very abundant during severe rickets, began very early in the healing process to be reduced in amount, its removal about keeping pace with the general reduction in the adjacent part of the metaphysis (Figs. 11, 12 and 13).

As in the metaphysis, the removal of osteoid in the end of the shaft was preceded by a dilute granular calcification which began at about Stage 2.

There also persisted a certain residue of osteoid on the calcified pre-rachitic trabeculae, in which, at about Stage 6, there began the formation of more dense deposits of calcium salts, producing an addition to the trabeculae by which the amount of bone was definitely increased (Figs. 13 and 14). These trabeculae at Stages 7 and 8 became quite robust, in contrast with the more slender ones in the adjacent metaphysis, and by them the end of the pre-rachitic shaft may be located (Fig. 14).

During the few later stages of healing, continued adjustment of trabeculae caused this difference to disappear, so that the boundary line between the pre-rachitic shaft and the rachitic metaphysis could no longer be recognized (Figs. 14 and 15). Thus the re-

organized tissues of the metaphysis-cartilage region have developed into a new end of the shaft, which terminates in a normal manner against the restored epiphyseal cartilage (Text-Fig. 1 B and C).

The Correction of Cupping: As the epiphyseal cartilage became restored to normal thickness and structure, the new end of the shaft assumed very much the normal form, having a definite convexity at the margin. At the same time the old concave termination of the shaft had been obliterated by its union with the metaphysis, as described above. Thus cupping was corrected by the substitution of a new end of the shaft for the old one, as seen in Text-Fig. 1 B and C and Figs. 7 to 9, and 13 to 15.

The Reorganization of the Epiphyseal Center

The epiphyseal portion of the bone was only mildly affected by rickets. The thickness of the trabeculae was only slightly increased, and there was only a thin layer of osteoid, not over $25\ \mu$ in thickness, formed upon them. The total amount of calcified material was definitely decreased, as shown by both silver and hematoxylin preparations. The marrow was not apparently changed in structure (*cf.* Figs. 5 and 9, and 11 and 16).

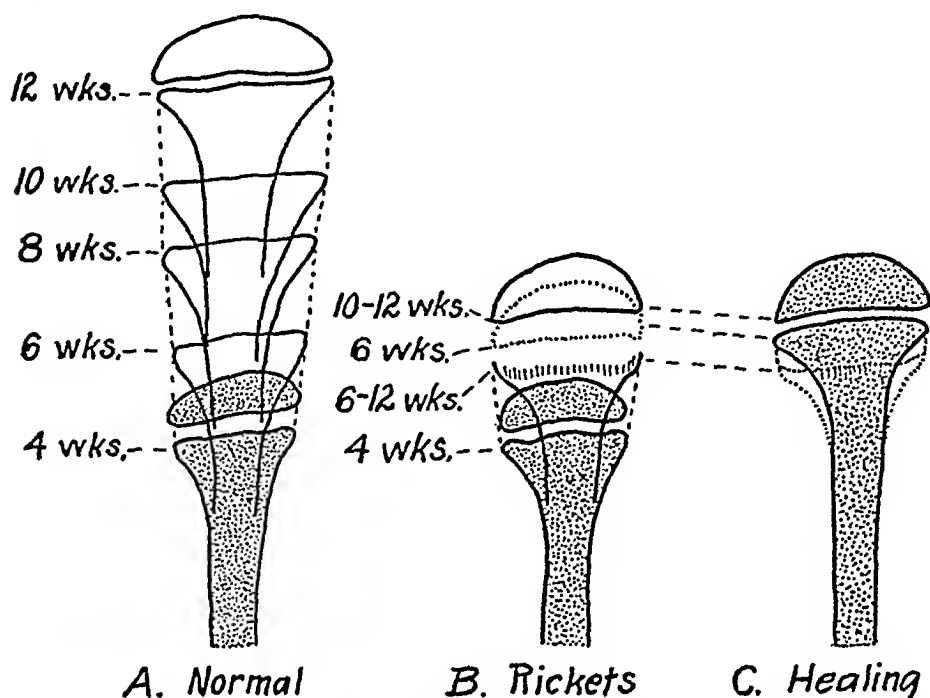
Healing in this region needs only brief mention. Calcification began to appear in the osteoid of the trabeculae in Stage 2. At about Stage 7 the amount of osteoid began to decrease and by Stage 11 the trabeculae appeared normal.

The Correction of the Shape of Rachitic Bones

Tubulation: Inasmuch as the ends of the diaphysis adjacent to the epiphyseal cartilages are greatly expanded in normal bones, it is necessary during the elongation of bones that there be a constant removal of recently formed bone from this region if the bone is to maintain its proper form. This process has been called "tubulation" by Jansen (Harris, 1933). The need for this removal becomes evident when we superpose X-ray photographs or scale drawings showing successive stages of a growing bone (Text-Fig. 1 A).

The Mechanism of Normal Tubulation: Our observations on the bones of healthy young rats show clearly that in the expanding curved portion of the end of the diaphysis, just below the epi-

physeal cartilage, there is no cortical layer of bone, but the parallel trabeculae end freely in the periosteum (Figs. 16 and 23). On the ends of these trabeculae there are many large osteoclasts. It is evident that by the continued removal of bone from the ends of



TEXT-FIG. 1. Outline drawings showing growth and tubulation of the normal and rachitic tibia from age 4 weeks to 12 weeks. Based on X-ray photographs enlarged to scale.

A = normal tibia, showing positions of end of shaft at biweekly intervals. The broken line bounds the area which must be removed in the normal process of tubulation.

B = rachitic tibia, showing limited growth during severe rickets. The shaft ceased to elongate after about 2 weeks on the rachitogenic diet (at age 6 or 7 weeks), due to cessation of normal cartilage removal. After this time the bone continued to elongate up to about 10 weeks by continued thickening of the epiphyseal cartilage. Tubulation ceased at about 6 weeks.

C = healing tibia, showing condition at end of healing process but before bone has resumed growth. New end of shaft has been formed by reorganization of thickened cartilage and rachitic metaphysis. Dotted lines bound areas removed in correcting the shape of the bone and the reestablishment of tubulation.

these trabeculae the characteristic curvature of this region of the shaft may be maintained and the tubular form of the shaft assured.

Interference with Tubulation During Rickets: During the development of rickets, osteoid was deposited in a thick layer under the periosteum overlying the free ends of the trabeculae in the

curved region of the shaft adjacent to the epiphyseal cartilage (Figs. 5, 11 and 24). At the same time the osteoclasts disappeared from the ends of the trabeculae. Thus tubulation ceased and the accumulation of osteoid added to the bulk of the bone in this region.

The Form of the Head of the Rachitic Bones: The rachitic bones continued to elongate for some time after the cessation of tubulation, the elongation being due to the continued thickening of the epiphyseal cartilage, even though none of it became incorporated in the end of the shaft proper (Text-Fig. 1 B). The result was that the portion added to the length of the bone after the cessation of tubulation had a rather uniform thickness equivalent to that at the level of the epiphyseal cartilage, or in some bones a gently tapered form, due to moderate increase in the diameter of the epiphyseal cartilage even during severe rickets (Text-Fig. 1 B, and Figs. 1, 5 and 11).

We have made numerous measurements on X-ray films of the bones at the wrist and knee and find no enlargement of the calcified structures in rachitic rats at either place. Nor do microscopic sections show any excessive amounts of uncalcified tissues at joints. From these observations we conclude that the unusual prominence of the joints in living rachitic rats is due to an undersized condition of the limbs adjacent to the joints rather than an enlargement of the joint.

The Correction of the Rachitic Form: At about Stage 2 there began to appear clouds of calcium granules in the osteoid covering the ends of the trabeculae in the curvature of the end of the shaft, just as similar deposits were forming in osteoid throughout the metaphysis and end of the shaft. At about Stage 5 the removal of this osteoid was under way, and by Stage 8 it had been completed (cf. Figs. 13 and 14). After the removal of the osteoid, osteoclasts reappeared upon the calcified trabeculae and the correction of shape then progressed steadily by the removal of calcified material (Figs. 1, 14 and 15). In our scale of healing Stage 12 has been defined as that at which the form of the bone no longer shows the rachitic deformity in X-ray photographs. It is of interest to note that by the time the external form had been corrected, the reorganization of the internal structure had also been brought to practical completion.

The Reorganization of the Marrow

The Marrow in Healthy Bones: In general there are four main functions performed by marrow: (1) osteogenesis, (2) hematogenesis, (3) conduction, and (4) storage, with definite structural components for each function. In the bones of healthy young rats the osteogenetic marrow is found conspicuously in the spongiosa of the shaft adjacent to the epiphyseal cartilage, but it occurs also on surfaces of bone trabeculae everywhere. Its various structural elements include conspicuously the osteoblasts and the osteoclasts. It is concerned with the removal of the epiphyseal cartilage and with the deposition and removal of bone tissue. The periosteum shares in the osteogenetic function.

The hematogenetic marrow in the tibia of young rats extends to within 300 to 600 μ of the epiphyseal cartilage, occupying the medullary cavity and the larger spaces in the spongiosa. The conducting function is accomplished by the abundant vascular channels, the walls of which are composed of little more than endothelium. In young rats the storage function is represented by only a few adipose cells.

The Marrow During Rickets: Though there is considerable variation in amount and nature of marrow during rickets, the following statements, we believe, express average conditions fairly well.

When rickets had developed to the extent that osteoid instead of bone was being deposited upon the trabeculae, the removal of material from the trabeculae ceased, or at least deposition was greatly in excess of removal. At this time osteoclasts nearly disappeared but numerous osteoblasts continued actively to deposit osteoid. Thus the trabeculae became greatly enlarged and the marrow spaces correspondingly reduced in size. Finally the osteoblasts and most of the undifferentiated marrow cells disappeared, and little was left of the marrow but endothelial vascular channels whose walls were nearly or quite in contact with the osteoid walls within which they lay (Fig. 17). There thus remained scarcely more than the conducting elements of the marrow. This condition was observed both in the metaphysis and in the adjacent end of the shaft.

During the course of rickets the hematogenetic marrow underwent little apparent change, an observation in harmony with the

findings of Sure and Kik (1931) to the effect that rickets produced no noteworthy interference with the process of hemopoiesis. In severe rickets this type of marrow approached rather closely to the end of the shaft, but did not enter the rachitic metaphysis.

The Marrow During the Healing of Rickets: Early in the healing process there appeared numerous large osteoclasts, most abundantly near the cartilage-metaphysis junction, but also generally distributed throughout the metaphysis and the spongiosa of the shaft. They appeared in the scant connective tissue between the endothelial blood channels and the osteoid. In some rats we have seen a considerable abundance of osteoclasts, apparently removing osteoid, even before there was any evidence of calcification. Osteoclasts continued to be very abundant through Stages 2 and 3, but by Stage 4 there was a decrease in their number. By that time the marrow spaces throughout the metaphysis and in the end of the shaft had become considerably larger and the amount of connective tissue surrounding the vascular channels had increased (Figs. 4, 17, 18 and 22).

During the early reorganization of the metaphysis, osteoclasts were especially abundant in the zone of calcified osteoid, the zone where the removal of osteoid was going on most rapidly. When the epiphyseal cartilage began to undergo rapid reduction in thickness, at about Stage 9, osteoclasts became abundant upon it, where they occupied characteristic positions on the ends of projecting calcified trabeculae.

The cellular elements responsible for the deposition phase of osteogenesis, the osteoblasts, first become common at about Stage 2 in Zone 1, the marrow zone of calcified cartilage trabeculae (Figs. 18 and 22). With the widening of this zone, osteoblasts spread throughout the metaphysis, and at the same time become abundant in the areas reclaimed from the projecting cartilage masses, and in the shaft. Thus the constructive elements of the marrow become widely established.

Hematogenic marrow began to develop in the end of the shaft adjacent to the metaphysis during Stages 5 and 6. It appeared among the connective tissue cells surrounding the vascular channels of the larger marrow spaces. In the metaphysis during Stages 5 and 6 there were formed a number of excessively large marrow spaces (Fig. 13). In them the vascular channels became greatly

distended, with very little connective tissue between their endothelium and the surrounding osteoid. It was in the connective tissue of these large spaces, during Stages 5 to 7, that we first observed hematogenous marrow in the metaphysis, at first in small amounts, but later increasing considerably, with a corresponding reduction in the size of the blood vessels. In the later stages of healing it advanced to its normal distance from the epiphyseal cartilage. At first it was in rather dilute form, and not until about Stage 12 did it attain normal density. Adipose cells appeared for the first time in this marrow at about Stage 12. So it was that by the time the bone and cartilage had attained normal form and structure, the marrow had also attained rather complete reorganization.

OBSERVATIONS ON OTHER BONES

In the distal end of the femur the observed details were very much like those described for the tibia, though they were less easily studied on account of the convoluted form of the epiphyseal cartilage. The distal ends of the radius and ulna differed from the proximal end of the tibia in that during severe rickets the persisting thick cartilage usually had the form of several projecting tongues instead of one large mass. Healing began in the metaphysis as it did in the tibia and spread toward the shaft and also laterally into the projecting cartilage masses, and later toward the epiphysis into the thinner portion of the cartilage. The general picture during healing, as shown both by X-ray and by microscopic study, was essentially the same as in the head of the tibia.

GENERAL RÉSUMÉ OF THE HEALING PROCESS

In the healing process of rachitic bones, as seen in the head of the tibia, there were two rather distinct phases. In the first phase, including Stages 1 to 6, there was accomplished a rather rough preliminary internal reorganization, involving in the main the removal of excess osteoid and important changes in the marrow. In the second phase, Stages 7 to 12, there took place a refinement of structural detail, and the correction of the shape of the bone, brought about in part by a continuation of certain of the processes initiated during the first phase.

The preliminary reorganization was accomplished largely in

TABLE II
General Sequence of Events in Healing

The two general phases of healing	Preliminary reorganization											Refinement of details				
	1	2	3	4	5	6	7	8	9	10	11	12				
Stages of healing as shown by X-ray photographs																
Provisional calcification of osteoid in metaphysis	x	X	X	X	X	X	X	X	X	X	X	X				
Calcification of cartilage trabeculae in metaphysis	x	X	X	X	X	X	X	X	X	X	X	X				
Calcification of thick parts of epiphyseal cartilage	—	X	X	X	X	X	X	X	X	X	X	X				
Removal of osteoid in metaphysis and shaft	x	X	X	X	X	X	X	X	X	X	X	X				
General reorganization of osteogenetic marrow	x	X	X	X	X	X	X	X	X	X	X	X				
Osteoclasts abundant in metaphysis and shaft	x	X	X	X	X	X	X	X	X	X	X	X				
Trabeculae rapidly changing shape and attitude	:	X	X	X	X	X	X	X	X	X	X	X				
Trabeculae undergoing continued but slower correction	—	—	—	—	—	—	X	X	X	X	X	X				
Hematogenetic marrow developing in metaphysis	—	—	—	—	—	—	X	X	X	X	X	X				
Osteoid being removed from expanded surface of shaft	—	—	—	—	—	—	X	X	X	X	X	X				
Shape of bone being corrected by removal of trabeculae	—	—	—	—	—	—	X	X	X	X	X	X				
End of pre-rachitic shaft becomes indistinguishable	—	—	—	—	—	—	—	—	—	—	—	—				
Permanent dense calcification of bone trabeculae	—	—	—	—	—	—	—	—	—	—	—	—				
Calcification zone of epiphyseal cartilage established	—	—	—	—	—	—	—	—	—	—	—	—				
Normal removal of epiphyseal cartilage resumed	—	—	—	—	—	—	—	—	—	—	—	—				
Epiphyseal cartilage restored to normal thickness	—	—	—	—	—	—	—	—	—	—	—	—				
Mitosis in epiphyseal cartilage probably resumed	—	—	—	—	—	—	—	—	—	—	—	—				
Cupping of end of shaft becomes corrected	—	—	—	—	—	—	—	—	—	—	—	—				
Divergence of bone trabeculae becomes corrected	—	—	—	—	—	—	—	—	—	—	—	—				

X = rapid progress of reorganization; x = less rapid progress; : = slow progress.

reverse direction, that is progressing from the cartilage toward the shaft, whereas in the second phase the direction of progress was again restored to normal, that is from the shaft toward the epiphysis. The general progress of healing is shown in Table II.

The Normality of our Healed Rachitic Bones: Comparison of Figures 15 and 16 shows how close may be the resemblance between a rachitic bone after restoration by the use of irradiated ergosterol or cod liver oil and one from a rat that never had rickets. Such excellent restoration is typical of our results, though in most such cases it seemed that calcification hardly came up to normal.

The Calcification Pattern During Healing: It has frequently been stated (Hess, 1929; Harris, 1933, and others) that in the healing of rickets calcification begins in the epiphyseal cartilage at the level where it would have been had rickets not developed. Our observations do not support this view. Had rickets not developed, calcification would have extended into the cartilage to include the whole zone of hypertrophied cells. We have always observed calcification to begin in the metaphysis adjacent to the cartilage, and not until well toward the completion of healing did it reach the level in the cartilage where it would have been had rickets not developed.

It is of interest to note also, that, as generally observed, calcification at first spreads toward the shaft in a direction opposite to that followed in healthy bones, and only in later healing stages is the normal direction resumed. The cause for this peculiar gradient of calcifying power is not evident. It is also of interest that the same order is followed by calcification of excised rat bones *in vitro*, as shown by numerous published illustrations.

SUMMARY

1. Rickets was produced by the Steenbock-Block diet; healing was brought about mainly by the administration of irradiated ergosterol.
2. Healing is described in detail from study of hematoxylin-eosin sections, silver preparations, and frequent X-ray photographs.
3. The twelve X-ray stages of Bourdillon are used as a scale for measuring the progress of healing.
4. The first indication of healing was calcification in the rachitic

metaphysis close to the edge of the epiphyseal cartilage, whence it spread, first through the metaphysis toward the shaft and later into the cartilage toward the end of the bone.

5. The preliminary reorganization of the metaphysis involved: provisional dilute calcification of the osteoid; calcification of the exposed cartilage trabeculae; increase in amount of marrow; and its restoration to normal structure. These changes restored a somewhat normal configuration of trabeculae by exposing the rather parallel cartilage trabeculae. The end of the shaft undergoes much the same changes.

6. At the same time the projecting cartilage masses became calcified and were partly removed, leaving calcified trabeculae very similar to those in the metaphysis.

7. During the latter half of healing the shape of the bone became corrected; hematogenous marrow formed in the new end of the shaft which had been formed from the metaphyseal region; cupping was corrected, the entire epiphyseal cartilage became normal in thickness and structure; and the reorganized trabeculae attained normal dense calcification.

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DESCRIPTION OF PLATES

Numerals, 1, 2, 3 (except in Fig. 1) indicate the three zones of the healing metaphysis as follows: 1 = zone of calcified cartilage trabeculae; 2 = zone of calcified osteoid; 3 = zone of uncalcified osteoid and cartilage.

Ca 1 = calcium deposit in edge of metaphysis, the "line" shown in silver preparations; Ca 2 = an unusual calcium deposit in metaphysis; C C = calcified cartilage trabeculae (in Zone 1); C O = calcified osteoid (in Zone 2); D = diaphysis or shaft; Ep = epiphyseal bone center; Ep C = epiphyseal cartilage; G = granular calcium deposit; H₁, H₂, H₃, etc., = healing stages; N = bone from normal rat; O = osteoid; Obl = osteoblasts; Ocl = osteoclasts; P = periosteum; R = bone from rat with acute rickets; U M = uncalcified rachitic metaphysis.

PLATE 53

FIG. 1. X-ray photographs of head of tibia showing our interpretation of the twelve healing stages of Bourdillon *et al.* Position of bones when rat lies relaxed under light ether anesthesia. N = normal bone; R = rachitic bone; 1, 2, 3, etc., the twelve healing stages. Reproduced from X-ray films. $\times 2$.

FIG. 2. Vertical section through surface of normal bone trabecula which is growing rapidly. Shows osteoblasts (Obl) under which is a layer of osteoid (O) 8 μ thick. Beneath this a layer of granular calcium (G) which grows more dense with depth. This deeply stained layer blends into the general mass of bone which stains less deeply and does not show granular structure. $\times 900$.

FIG. 3. Area in rachitic bone at Stage 5 of healing. Dark areas represent pre-rachitic bone, stained deeply with hematoxylin. Osteoid (O) is pale, and recently formed calcium granules (G) are shown in varying density. $\times 900$.

FIG. 4. Area in metaphysis of bone at healing Stage 2. Zone 1 shows calcified cartilage trabeculae (C C), from which most of the osteoid has been removed. Zone 2 shows calcification in osteoid (C O) but none in the contained cartilage trabeculae. Shows nature of restored marrow clearly. $\times 450$.

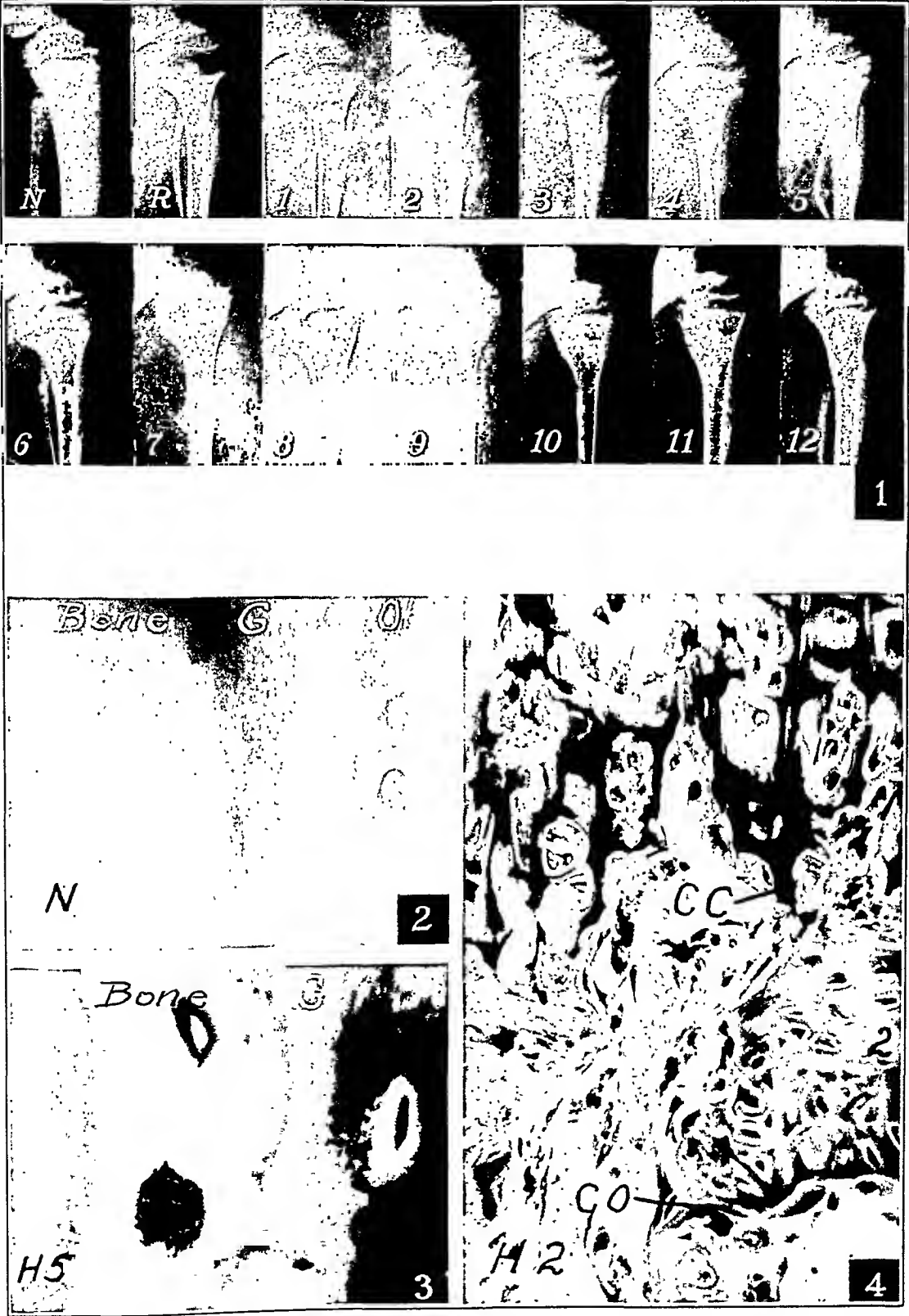


PLATE 54

FIGS. 5, 6, 7, 8. Head of tibia, silver preparations (line test). Calcified areas colored dark. $\times 15$.

FIG. 5. Severe rickets, showing epiphyseal ossification (Ep), thick and thin portions of epiphyseal cartilage (Ep C); uncalcified rachitic metaphysis (U M); and diaphysis (D).

FIG. 6. Healing Stage 1. Structures as in Fig. 5, except for deposition of calcium line (Ca_1) in border of metaphysis, and a second rather unusual area of calcification (Ca_2) deeper in the metaphysis.

FIG. 7. Healing Stage 2. The calcified area has extended entirely across the bone, both in the metaphysis and in the thickened part of the cartilage. It has also become thicker by expansion toward the shaft. The three zones of the healing metaphysis are shown. Zone 1 of calcified cartilage trabeculae; Zone 2 of calcified osteoid masses; and Zone 3, the uncalcified portion.

FIG. 8. Healing Stage 6. Calcification has extended entirely across the metaphysis to the end of the shaft obliterating Zone 3. It has also invaded most of the thickened cartilage area. Zone 1 has become greatly widened, but is no longer sharply distinct from Zone 2. The trabeculae have attained approximately their normal configuration. The large uncalcified area in the center of the bone is unusual. Variations in pattern of healing are by no means uncommon. The form of the bone is still rachitic.

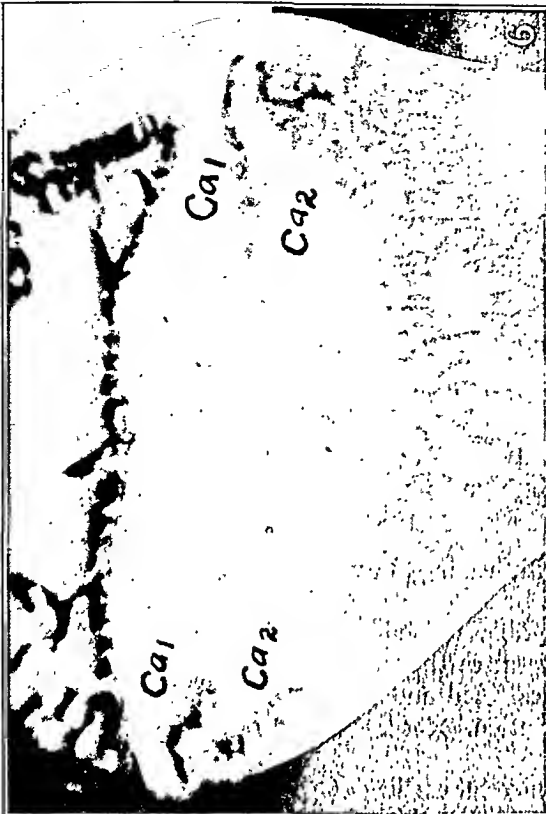


PLATE 55

FIGS. 9, 10. Silver preparations of head of tibia, continuing the series begun on Plate 54. $\times 15$.

FIG. 9. Healing Stage 12. The epiphyseal cartilage has been reduced to normal thickness and the entire pattern of calcification has progressed to about normal amount and distribution. Former rachitic metaphysis and end of pre-rachitic shaft have united to form new end of shaft with rather normal spongiosa. Cupping has been corrected, and shape of bone has become normal.

FIG. 10. Normal tibia, for comparison with healing Stage 12, shown in Fig. 9.

FIGS. 11, 12. Sections through head of tibia stained with hematoxylin and eosin. The dark color of the epiphyseal cartilage does not indicate calcification. The distinction between calcified and uncalcified cartilage is seen clearly under microscopic observation. $\times 15$.

FIG. 11. Severe rickets (*cf.* Fig. 5). Epiphyseal cartilage (Ep C) showing reduced region, still abnormally thick, and the thickened area still resting upon the end of the pre-rachitic shaft. Note entire absence of calcification in the metaphysis (U M), and the abundance of osteoid and small amount of calcification in the end of the shaft (D). Note extreme rachitic form of the head of the bone.

FIG. 12. Healing Stage 2. The most conspicuous healing change is the development of the zone of calcified cartilage trabeculae (Zone 1) in the border of the metaphysis. Zones 2 and 3 are not as clearly shown as in the silver preparation (*cf.* Fig. 7).

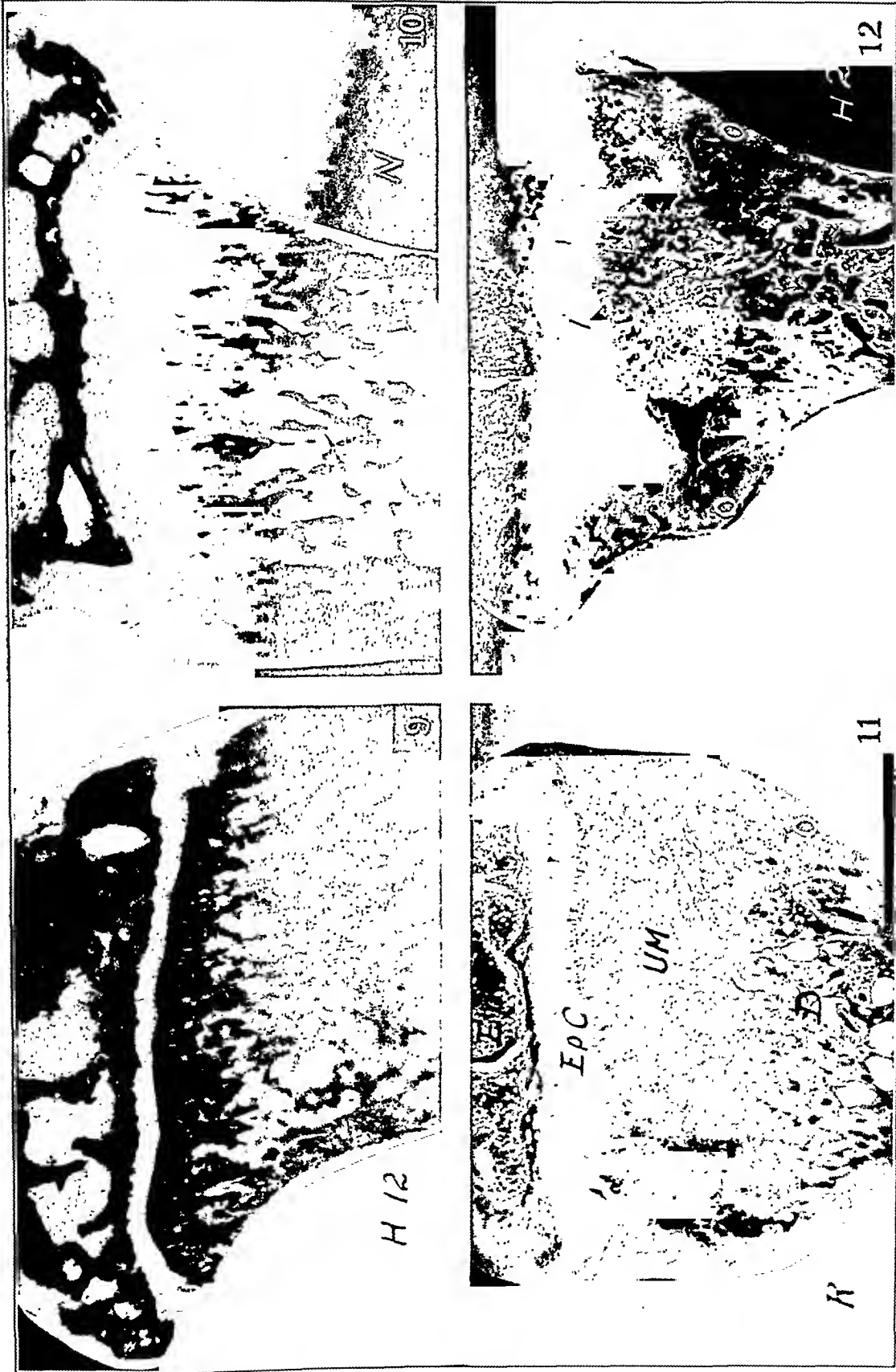


PLATE 56

FIGS. 13, 14, 15, 16. Sections stained with hematoxylin and eosin. Continuation of series begun on Plate 55. $\times 15$.

FIG. 13. Healing Stage 6 (*cf.* Fig. 8). Thick part of cartilage largely removed (represented only by isolated remnant adjacent to end of shaft). Trabeculae have become more parallel by removal of excess osteoid, allowing underlying pattern of cartilage to dominate. Zonation no longer distinct, due to spread of Zone 1 toward shaft. Dilute provisional calcification of osteoid has spread to end of shaft. Osteoid covering still present on curvature of head.

FIG. 14. Healing Stage 8. Thick part of cartilage completely removed, but entire remaining portion of cartilage is still abnormally thick. Trabeculae approaching normal configuration, though the radial divergence caused by a similar divergence of the cell columns in the thickened cartilage still shows plainly. Calcification in cartilage trabeculae is strong, but osteoid covering is still not fully calcified. Larger calcified trabeculae in end of shaft still differentiate it from metaphysis. Osteoid has been removed from surface of head but rachitic shape still persists. Portion to be removed in correction of shape outlined in broken lines. Hematogenous marrow is developing in large marrow spaces in lower part of metaphysis.

FIG. 15. Healing Stage 12 (*cf.* Fig. 9). Epiphyseal cartilage reduced to normal thickness. Trabeculae have assumed nearly normal configuration and are well calcified. The radial divergence has been nearly corrected. Shaft and metaphysis are fully united. Cupping has been corrected and shape of bone restored to normal by reestablishment of tubulation process.

FIG. 16. Normal bone, for comparison with Fig. 15. The lighter coloring of the epiphyseal cartilage in this figure is due not to a difference in the cartilage, but to the use of a different hematoxylin stain from that used in other sections.

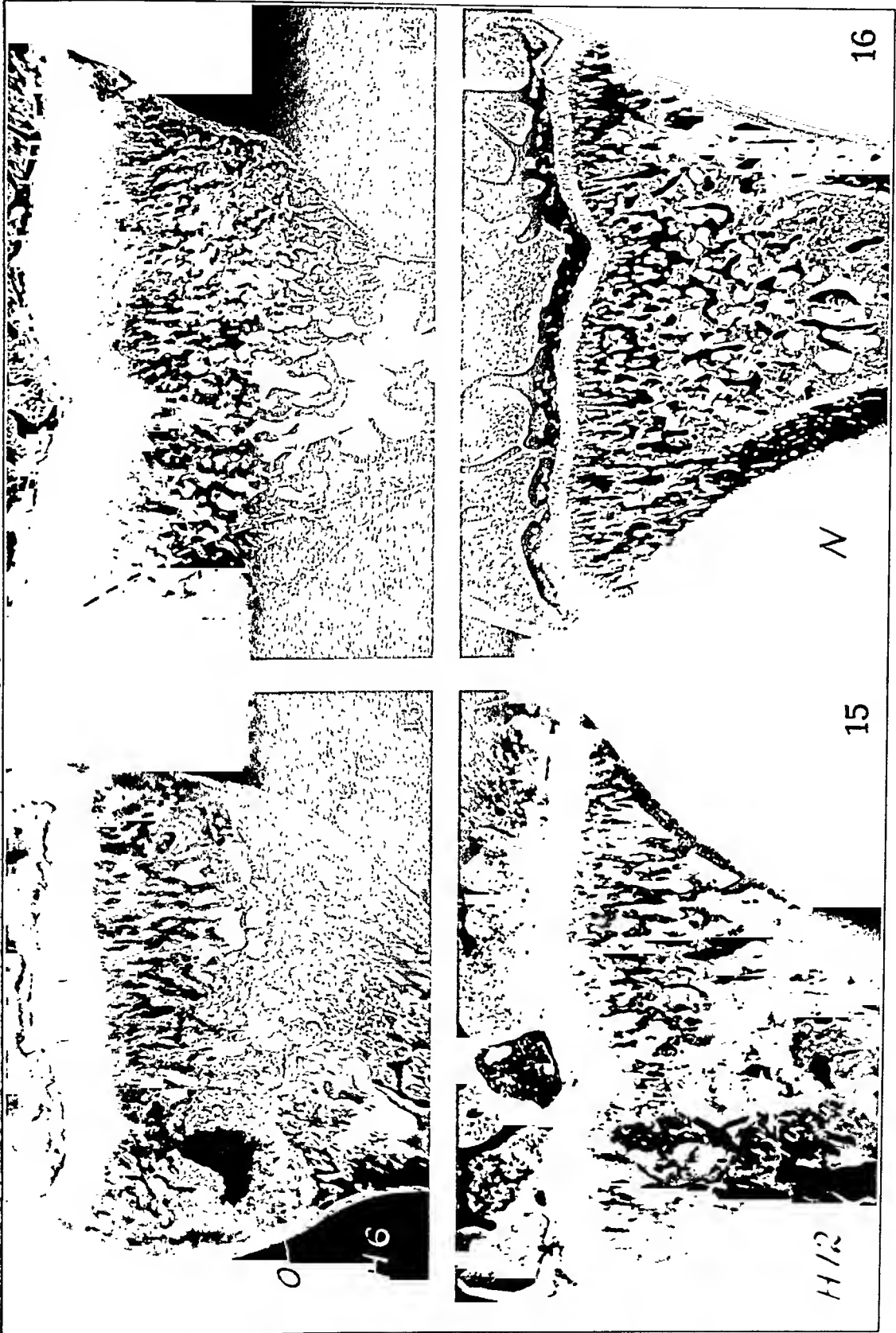


PLATE 57

FIGS. 17, 18, 19, 20. Series of microphotographs showing healing process in edge of rachitic metaphysis. Sections stained with hematoxylin and eosin. $\times 100$.

FIG. 17. Severe rickets. Shows trabeculae of uncalcified rachitic metaphysis, composed of masses of osteoid enclosing uncalcified cartilage remnants. The marrow in the greatly reduced spaces is almost wholly vascular. At border between cartilage and metaphysis there is considerable accumulation of osteoid, indicating well advanced rickets.

FIG. 18. Healing Stage 2 (*cf.* Figs. 7 and 12). Shows early healing region (Zone 1) about $100\ \mu$ wide, where excess osteoid has been removed from the cartilage trabeculae, which have subsequently become calcified. In this zone the marrow spaces have greatly increased in size, in Zones 2 and 3 moderately so. In all zones the marrow has become less vascular and more cellular. Zone 2 shows calcification of osteoid, though not strongly.

FIG. 19. Healing Stage 4. Shows further progress of changes begun in Fig. 18. Zone 1 is much wider than in Stage 2 ($400\ \mu$). Calcification shows strongly in cartilage trabeculae of Zone 1 and in osteoid masses of Zone 2.

FIG. 20. Healing Stage 6 (*cf.* Figs. 8 and 13). Zone 1 has become much widened by removal of osteoid and calcification of cartilage trabeculae. Configuration of trabeculae approaching normal, but osteoid layer is still mostly free from calcification.

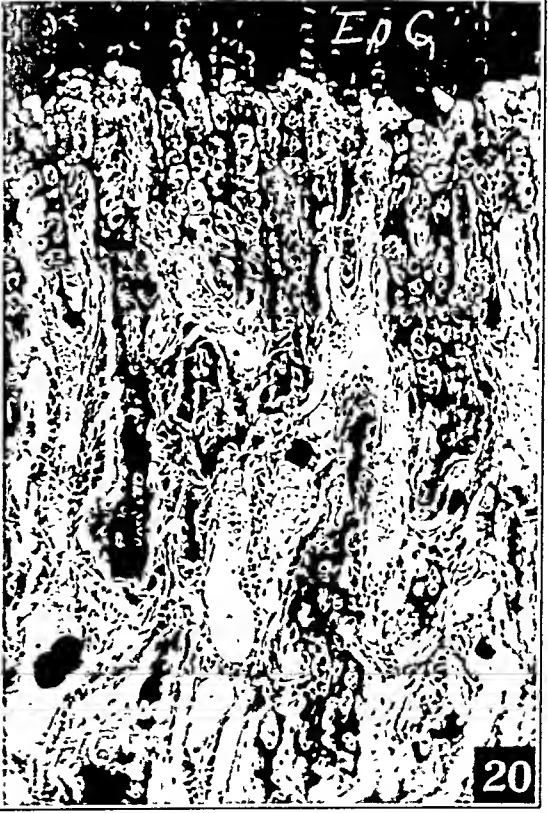
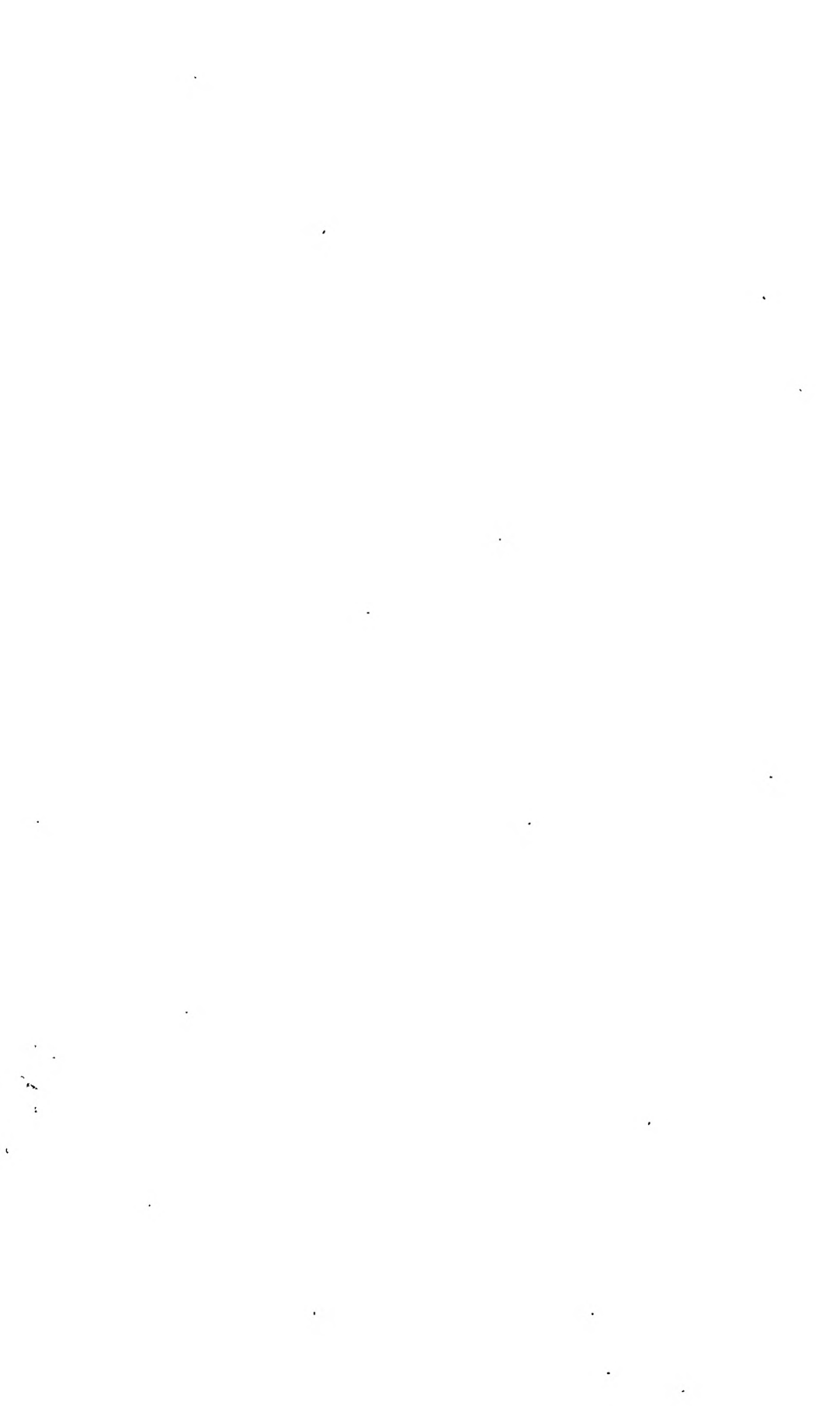


PLATE 58

- FIG. 21. Continuation of series on Plate 57. Healing Stage 12. Epiphyseal cartilage has been reduced to normal thickness and has become normally calcified. Calcification and form of bone trabeculae have become normal. Hematogenous marrow has developed well toward cartilage. $\times 100$.
- FIG. 22. Healing Stage 6. Hematoxylin and eosin stain. Chosen to show details of calcification in Zone 1 and edge of epiphyseal cartilage. Note that calcification is in two narrow bands on the two sides of the trabeculae and that it extends into the cartilage only about one lacuna. Osteoid on cartilage trabeculae not calcified at this stage. Note also normal appearance of marrow with well developed osteoblasts. $\times 450$.
- FIG. 23. Normal tibia. Hematoxylin and eosin stain. From curved part of bone below epiphyseal cartilage to show mechanism of tubulation process. Note lack of cortical bone, and presence of osteoclasts on ends of trabeculae ending in periosteum. $\times 100$.
- FIG. 24. Severe rickets. Region corresponding to Fig. 23. Thick osteoid layer ($300\ \mu$) has covered ends of trabeculae, osteoclasts have disappeared; bone removal in this region has ceased, which means that tubulation is not possible (*cf.* Fig. 11). $\times 100$.





PARS INTERMEDIA BASOPHIL ADENOMA OF THE HYPOPHYSIS *

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Recent reviews of Cushing's pituitary basophilism (Tesseraux,¹ Brauer,² Pardee,³ Bland and Goldstein,⁴ and others) indicate that the rôle of the hypophysis in this syndrome is far from being settled. There are certainly many cases of this type in which no basophil adenoma is demonstrable, and some without any hypophyseal tumor of any kind. The 2nd case reported by Ulrich⁵ is an example where no evidence of adenoma could be found in any organ. Practically all cases, however, show some hyalinization of the basophils as described by Crooke.⁶ Statements to the effect that there is hyperplasia of the basophils of the anterior lobe, but no distinct adenoma, must be taken with considerable reservation because of the failure to recognize the irregular distribution of these cells normally, the lack of quantitative methods (Rasmussen⁷) and insufficient examination of the gland.

Many basophil adenomas have been found without being accompanied by so-called pituitary basophilism (Kraus,⁸ Costello,⁹ and Susman¹⁰). Close¹¹ reported a case where the only symptom common to pituitary basophilism was high blood pressure. The syndrome has occurred in cases purported to show acidophil adenomas of the hypophysis (Reichmann,¹² Korschegg,¹³ and Horneck¹⁴), and where the hypophyseal tumor was strictly chromophobic (Fuller and Russell¹⁵), or chromophobic with a few scattered basophils (Crile, Turner and McCullagh¹⁶). The case reported by Bettoni,¹⁷ in which the anterior lobe was almost completely occupied by a chromophobic adenoma, probably belongs in this general category. In a number of instances it has been impossible to be definite as to the basophilic character of the tumor. In the 1st case reported by Ulrich,¹⁸ slight indications of the basophilic character of the granules could be obtained only with great effort and then only in a few of the larger cells. In all recent extensive lists of cases there are several in which the type of adenoma was not clearly established.

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A high percentage of cases with the syndrome of pituitary basophilism have either an adrenal cortical tumor or distinct hypertrophy of the adrenal cortex. McQuarrie, Johnson and Ziegler¹⁹ have emphasized the fact that the electrolyte pattern of the plasma in most respects may be diametrically opposite to that found in Addison's disease, and hence suggest "hypercorticoadrenalism" as the most expressive name of the syndrome. This is further strengthened by the studies of Jores,²⁰ and by Anderson and Haymaker,²¹ who found evidences of increased adrenal cortical hormone in cases of Cushing's disease. Bauer²² is rather emphatic that the syndrome is a form of interrenalism, while Kraus⁸ argues that both the hypophyseal and adrenal changes are compensatory in nature, secondary to fat metabolism and associated metabolic disturbances. Various differential diagnoses between adrenal cortical hyperfunction, basophil adenoma of the hypophysis and arrhenoblastoma of the ovary have been attempted (Goldzieher and Koster²³), but great difficulty still exists as is shown by a case (Norris²⁴) presenting a malignant ovarian tumor. The report by Walters, Wilder, and Kepler²⁵ on the suprarenal cortical syndrome represents the situation well. A very recent case (Pons and Pappenheimer²⁶) with the "classic" features of so-called pituitary basophilism showed neither a basophil adenoma nor hyalinization of the basophil cells and was finally designated "renal hyperparathyroidism."

It is, therefore, advisable to record all basophil adenomas of the hypophysis so that their significance may be better understood. Apparently it is necessary to allow for a fair percentage of chance coincidence. The cases reported here are of special interest because the adenomas appear to have arisen from the same source as the basophilic cells that normally invade the neural lobe. Since these invading basophils normally differentiate from the pars intermedia (Rasmussen,²⁷ Roussy and Mosinger,²⁸ Plaut,²⁹ and Lennon³⁰) we are apparently dealing with pars intermedia tumors. The migration of basophilic cells into the neural lobe has been greatly discussed in recent years, particularly in connection with hypertension (Cushing^{31, 32}). Ahlström,³³ Gómez Marcano,³⁴ and Leary and Zimmerman³⁵ present additional data that have been interpreted as supporting more than mere chance correlation between the invasion and hypertension. Yet Gómez Marcano does

not consider that a constant relation exists between the degree of basophilic invasion and the height of the blood pressure. Most observations, however, do not favor any relation (Kraus,³⁶ Spark,³⁷ Biggart,³⁸ Berblinger,³⁹ Butt and Van Wart,⁴⁰ Seecof,⁴⁰ Boyd,⁴⁰ Hawking,⁴¹ Scriba,⁴² Plaut,²⁹ Parsons,⁴³ and Rasmussen⁴⁴), although a few of these authors think there is some connection between the abundance of basophils in the *anterior* lobe and high blood pressure. Again, Kraus thinks this basophilic invasion may be related more directly to adiposity than to blood pressure, particularly in eclampsia.

In connection with diffuse basophilia of the anterior lobe and its relation to blood pressure, it should be noted that only in the reports by Hawking⁴¹ and Rasmussen⁴⁴ were the cells enumerated, and both these studies show that the increase in percentage of basophils in essential hypertension is not regular enough to be regarded as possessing etiological significance.

The following cases are presented as additional evidence that great caution is still necessary in estimating the effects of basophil adenomas of the hypophysis.

CASE REPORTS *

CASE 1: The tumor was found unexpectedly at autopsy in a well developed 77 year old white male who was admitted to the University of Minnesota Hospital May 10, 1937 because of nocturia, urgency, incontinence and loss of weight of 9 months duration, although he had had mild urinary symptoms for many years. Slowness of speech and comprehension had been evident for a year. Occasional edema of the ankles occurred. The blood pressure 1 year before admission was 260/90 mm. Hg. He had slight dyspnea but no other cardiorespiratory symptoms.

On admission the systolic blood pressure ranged from 200–260 mm. Hg., and the diastolic from 115–150 mm. Hg. An occasional extra systole was noted. Pitting edema of the ankles was present. The prostate was moderately enlarged. Residual urine amounted to 750 cc. and contained traces of albumin and numerous blood cells but no sugar. The blood showed 85 per cent hemoglobin, 10,300 leukocytes and 62 per cent neutrophils. The blood urea nitrogen was 22 mg.; phenolsulphonephthalein 44 per cent; Wassermann negative.

Within 2 days there developed thickness of speech, dropping of left side of mouth, deviation of the uvula and tongue, modified reflexes and

* We are indebted to the Department of Surgery, University of Minnesota, for permission to publish these cases.

weakness of the left extremities. The electrocardiogram indicated auricular fibrillation and ventricular extrasystoles. A cystostomy was done a week later and the prostatic condition greatly improved. Symptoms of cerebral thrombosis persisted and the patient died on the 18th day.

Autopsy Findings

The autopsy was performed 45 minutes after death. The body was emaciated, 171 cm. in length and weighed about 115 pounds. A subcutaneous lipoma 4 cm. in diameter was present in the left hypochondriac region. The heart weighed 430 gm. and showed slight left ventricular hypertrophy but no fibrosis of the myocardium. The mitral and aortic valves, coronary arteries and the root of the aorta showed moderate senile atheromatosis. The kidneys were quite smooth and weighed 130 and 150 gm. respectively. The parenchyma was pale and slightly fibrous. Microscopic sections revealed slight senile arteriosclerotic changes but no hypertensive changes. Moderate bilateral hydronephrosis and hydroureter were present. The bladder was markedly trabeculated. There was moderate hemorrhagic cystitis. The lateral lobes of the prostate were slightly hypertrophied, there was a fibrous median bar formation, and sections showed irregular nodular adenocarcinomas. The adrenals were normal. The left testis was markedly atrophic. The thyroid was small, brown and fibrous with an adenoma (3 by 3.5 by 4.5 cm.) at the left lower pole. This adenoma contained a cyst measuring 2 cm. in diameter with a calcified wall. The parathyroids were normal. No thymic tissue could be seen.

The blood vessels at the base of the brain were sclerotic and greatly reduced in size. After injection with formalin the brain was found on section to contain numerous small cystic areas in the region of the right internal capsule and a few in the left side. On the right was a fairly large area of softening involving the internal capsule and basal nuclei. Microscopic examination revealed no cellular reaction around these cavities. Some were of recent origin.

The upper aspect of the sella turcica appeared to be normal but dissection uncovered a tumor about 1.5 cm. in diameter adherent to the right side of the hypophysis and attached to its lower pole. The adenoma extended underneath the right internal carotid

artery (Fig. 1), and depressed the roof over the right side of the sphenoid sinus, but did not appear to have invaded the bone at any point. Discovery of this neoplasm stimulated a more careful examination of the body for further evidences of endocrine disorders, but the body build, muscular development and hair distribution were all normal. There was no evidence or history of obesity, purple striations or acromegaly. After serial sections of the hypophysis showed the tumor to be a basophil adenoma, the records were carefully checked but no signs or symptoms indicative of pituitary basophilism existed, except the high blood pressure.

The hypophysis was somewhat asymmetrical on account of the pressure of the tumor on the right side. The tumor was cut so that part of it remained attached to the hypophysis and the rest was removed with the internal carotid artery. Both blocks of tissue were fixed in formalin, cut at $5\ \mu$ and stained with the Mallory-Heidenhain and hematoxylin-eosin stains. A rather sharp boundary line, which gradually merged into the connective tissue capsule of the anterior lobe more anteriorly, separated the new-growth from the anterior lobe (Fig. 2). As the series of sections were followed toward the lower pole the tumor became denser, large nodules of strongly basophilic cells appeared (Fig. 3) and there was continuity with a great mass of similar cells that infiltrated the neural lobe (Fig. 3).

A careful study of serial sections indicates that the outgrowth has been from the lower pole of pars intermedia — a region where basophilic invasion of the neural lobe is prevalent. The cells in well preserved portions of the adenoma are identical with the cells not only in the adjacent neural lobe but also in remote regions of this lobe (Fig. 2) where they have unquestionably been derived in the usual manner from the pars intermedia. The cells are variable in size. None are excessively large. They are distinctly granular but only occasionally vacuolated. A high power view of the area marked + in Figure 3 is shown in Figure 5. Much of the tumor contains only small, irregular basophilic islands (the dark areas in Figure 4) in which the cells are degenerating and contain only a few basophilic granules. The intervening region consists of a loose network of connective tissue invaded by neutrophils and containing some scattered indifferent cells with little or no cyto-

plasm and cells in various stages of degeneration. In limited areas there is diffuse hemorrhage and fibrin formation. As a whole the tumor is poorly vascularized. There is a small indifferent staining region in the anterior lobe (Figure 2, just below the end of the leader to the anterior lobe) which appears to be degenerative. No hyalinization of the basophils is present.

Comment: Owing to the advanced age of this individual, one would hesitate to associate the high blood pressure with the basophilia. The probability of mere chance coincidence would appear to be too great. It should also be recalled that hypertension is not uncommon in acromegalia which is due to acidophil adenoma. Jéquier⁴⁵ found this to be true in over a third of the cases. Hypertension has been found in 60 per cent of acromegalic females over 40 years of age. The literature is well reviewed by Houssay.⁴⁶

CASE 2: A white female, 55 years of age, was admitted to the University of Minnesota Hospital Nov. 22, 1937, with a strangulated umbilical hernia. Because of the condition of the patient no physical findings, except those immediately connected with the hernia, were obtained. She was operated upon immediately. Some dark loops of intestine near the hernial sac became progressively better in color, showed active peristalsis, and were, therefore, returned to the abdomen and the hernia repaired. The systolic pressure was down to 70 mm. Hg. Transfusion of 700 cc. of blood and 800 cc. of normal saline brought the systolic pressure to 90 mm. Hg. where it stayed. The patient was cyanotic and was put in an oxygen tent but expired the same day.

Slightly less than a year preceding death the patient was examined by a local physician because of dyspnea and a fainting spell. Her weight at that time was 225 pounds and the blood pressure was 204/96. The lower parts of the legs were edematous. The urine was free from sugar and albumin. Six months previously she had been examined by another physician during an acute attack of influenza. The hypertension and dyspnea were evident at that time. The heart sounds were slightly irregular and rather weak. A moderate number of granular casts were present in the urine.

Obesity and hair on the face appeared shortly after the birth of her first child, 33 years ago. She had three normal children subsequently and continued to menstruate regularly till 51 years of age. She even menstruated once when 54 years of age.

Autopsy Findings

The autopsy was performed about an hour after death. The body was very obese, 168 cm. in length and about 210 pounds in

weight. The obesity was marked on the trunk and upper portions of the extremities. There was slight edema of the legs and numerous varicose veins. Papillomas up to 1 cm. in diameter were present on the right labium majus, the inside of the right thigh, the right nipple, and small ones were present on the upper eyelids. A heavy growth of thick stiff hairs was present around the mouth and on the chin. Purple striae were clearly evident on the abdomen. The outward features were characteristic of Cushing's pituitary basophilism so that the body was carefully examined for further evidences of this syndrome.

The umbilical hernia had been repaired and was in good condition and there was no peritonitis or ascites, but about 6 feet from the ileocecal valve was a segment of small intestine 10 cm. long which showed early gangrene. The abdominal subcutaneous fat was 6 cm. in thickness. The heart weighed 575 gm. There was marked ventricular hypertrophy on the left and slight hypertrophy on the right. The myocardium of the anterior portion of the left ventricle showed a moderate degree of old spotty fibrosis but no large infarction. The valves were normal. The foramen ovale was closed. Atherosclerosis was moderate in the coronary arteries and much of the aorta. There was a stone 7 mm. in diameter in the common bile duct and the gall bladder was tightly filled with about fifty stones. A moderate degree of fat replacement was evident in the pancreas. The kidneys were essentially normal on gross appearance but sections showed a moderate degree of hypertensive changes in the small arteries and arterioles. The adrenals were normal. The uterus weighed 225 gm. and contained about a dozen interstitial myomas up to 2 cm. in diameter. The cervix uteri was eroded and cystic. The uterine tubes and right ovary were normal. The left ovary was replaced by a multilocular cystic mass 7 by 8 by 10 cm. which contained clear yellow fluid. The wall of one of these cysts had a few early papillomatous growths. There were a few small cysts around the right ovary. At the lower pole of each lobe of the thyroid was an adenoma of the mixed type. The one on the left was 3 cm. in diameter and was calcified, while the one on the right was soft, pale in color, and measured 4 cm. in diameter. Sections of the thyroid indicated moderate atrophy and fibrosis with lymphocytic foci. The parathyroids were normal. No thymic tissue was visible.

The brain weighed 1210 gm. and showed nothing unusual beyond a moderate degree of sclerosis in the vessels at the base. The sella turcica was normal. The hypophysis weighed 0.682 gm. and except for a moderate degree of cupping on top gave no outward indications of any abnormality. It was fixed in formalin, sectioned serially and stained as in Case 1. A definite basophil adenoma about 3 mm. in diameter was found on the left side of the upper region of the pars intermedia and extending into the rim formed by the depression of the cerebral part of the upper surface of the gland (Figs. 7).

On microscopic examination the cells of the adenoma are found to be strongly basophilic, densely packed (Fig. 6), and to compress the upper left pole of the anterior lobe from which the adenoma is separated by a sharp boundary line in which are flattened colloid vesicles and other traces of pars intermedia (Figs. 7 and 8). At about the middle of the series it becomes less compact and merges into a stratum of basophilic cells which extends across the entire anterior portion of the neural lobe and enlarges at the right margin so that there is a more or less symmetrical bilobed basophilic area throughout the rest of the gland to the lower pole (Fig. 9). The cells of the adenoma proper (Fig. 6) present the structural features of the basophilic cells that normally migrate into the neural lobe. In the interior is an irregular fluid-filled space (Fig. 7) which has no definite wall. There are no indications of degeneration.

Numerous, small circumscribed masses of basophils (Fig. 10) are found throughout most of the anterior lobe, particularly in the upper part of the gland and near the pars intermedia. Larger areas of more diffuse basophilia are intermingled with the above so that there is an excess of basophilic cells in the anterior lobe also. An actual differential count of the cells of the anterior lobe showed that there were 39 per cent chromophobes, 30 per cent acidophils and 31 per cent basophils. The average percentage of basophils in females normally is about 7 per cent and they rarely reach 17 per cent.⁷ There is no hyaline change such as is almost always evident in pituitary basophilism. This hyalinization is regarded by Crooke as an expression of altered physiological activity, while Severinghaus⁴⁷ thinks it is a degenerating process.

Comment: In spite of the adenoma and the excess number of

basophils in both lobes of the hypophysis and the presence of several of the characteristics of Cushing's syndrome, there were no menstrual disturbances, glycosuria, osteoporosis, hypertrophy of the adrenal cortex, or hyaline change in the basophilic cells of the hypophysis. The obesity had existed for nearly 33 years. Crooke and Russell would exclude this from the list of true pituitary basophilism.

At least 2 other basophil adenomas apparently originating from the pars intermedia are on record — the Raab-Kraus case and Case 6 (fatal eclampsia) reported by Cushing — both described in detail by Cushing.³² They were wholly within the posterior lobe. A 3rd case, published by MacCallum, Futcher, Duff and Ellsworth,⁴⁸ is undoubtedly an anterior lobe tumor. It was strictly within the anterior lobe but posteriorly extended to the intermediate region. Their principal reason for considering it of pars intermedia origin was the mistaken idea that the copper hematoxylin method stains the basophils of the anterior lobe dark blue. The tumor by ordinary stains was basophilic but did not stain with copper hematoxylin. The facts are that it is the acidophils of the anterior lobe that stain deep blue with copper hematoxylin,⁴⁹ and neither the basophils of the anterior lobe nor those that arise from the pars intermedia take this stain strongly.

SUMMARY

Two cases of basophil adenoma originating from the pars intermedia are described. In 1 case the only symptom at all referable to the hypophysis was high blood pressure. On account of the old age of this individual (77 years) the association of the adenoma with the hypertension is questionable. In the 2nd case a number of the major characteristics of pituitary basophilism (adiposity, striae atrophicae, hirsuties, high blood pressure, florid face) were present. In neither case were there any hyaline changes in the basophils.

There was considerable diffuse invasion of the neural lobe by basophils in both cases. The great bulk of data, however, does not indicate that there is any direct significant correlation between this invasion and hypertension.

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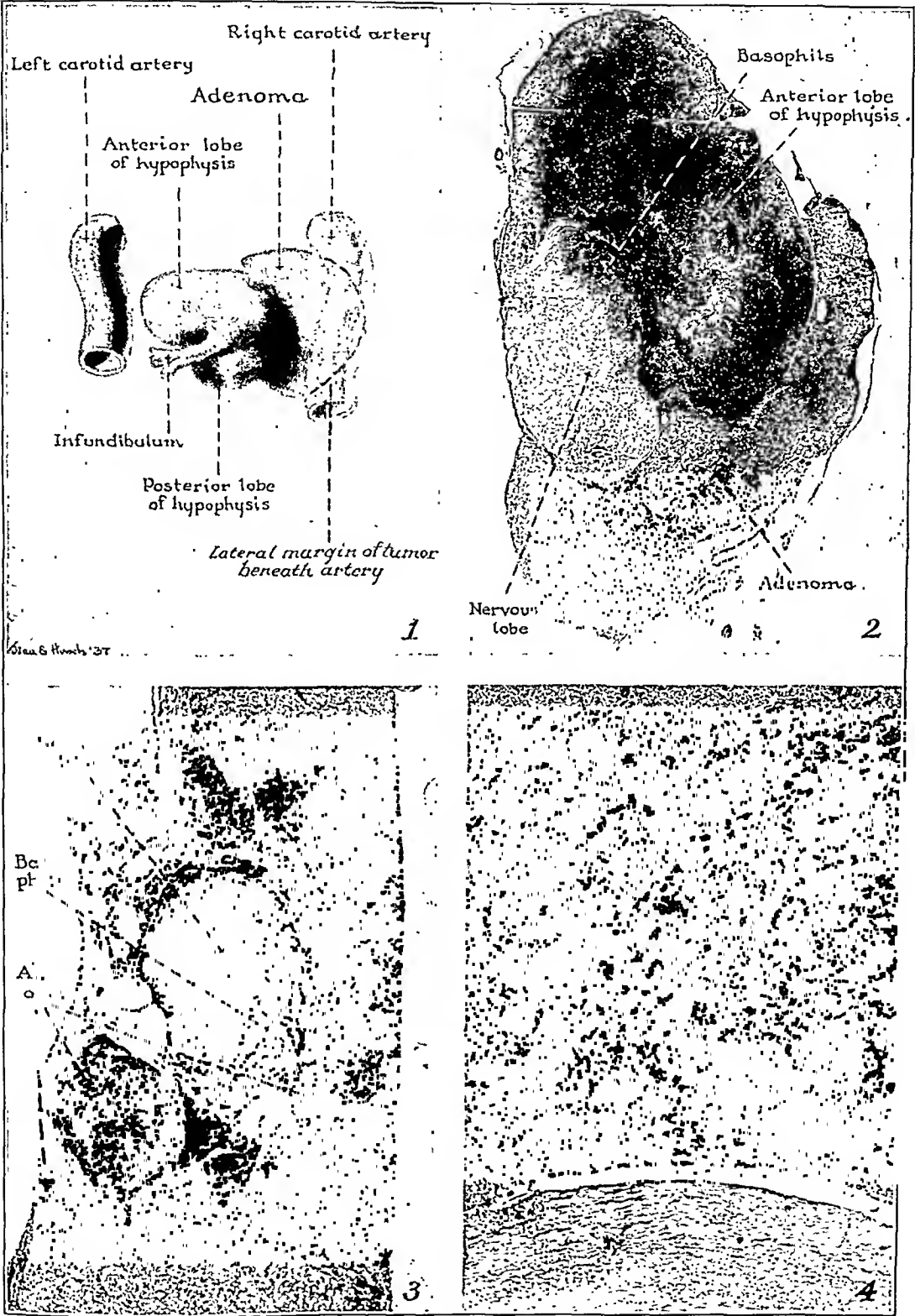
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DESCRIPTION OF PLATES

PLATE 59

- FIG. 1. Case 1. Drawing of the hypophysis and adenoma in relation to the internal carotid arteries. Viewed from above. The lateral margin of the tumor underneath the right carotid artery is indicated by a broken line.
- FIG. 2. Case 1. Microphotograph of a horizontal section through the hypophysis and part of the adenoma, which is closely applied to the right side of the hypophysis but sharply separated from the latter at this level. A large number of basophilic cells have invaded the neural lobe quite remote from the tumor. A small, circumscribed degenerating area is seen at the end of the leader in the anterior lobe.
- FIG. 3. Case 1. Microphotograph of a horizontal section through the lower pole of the hypophysis at about the middle of the adenoma which is continuous with the basophils of the pars intermedia, which completely encircle the neural lobe at this level. A conspicuous nodule of strongly basophilic cells in the adenoma is indicated by a white +. Except for such nodules the tumor is for the most part loose like the lower third of the figure. At slightly lower levels, where there is no longer any evidence of the neural lobe, the continuity between the adenoma and the pars intermedia is even more evident, but an illustration from this lower level would be less informative than the one here reproduced.
- FIG. 4. Case 1. Microphotograph at a higher magnification showing that portion of the tumor which is in contact with the internal carotid artery. The wall of the artery is shown in the lower part of the figure. The irregular scattered dark areas are mostly degenerating basophils.



Rasmussen and Nelson

Pars Intermedia Basophil Adenoma of Hypophysis

PLATE 60

- FIG. 5. Case 1. High power view of the area in Fig. 3 marked with a white +. The cells are variable in size and in degree of granulation and only occasionally vacuolated. In structure and staining reaction these cells are indistinguishable from the basophils that normally differentiate from pars intermedia and more or less invade the neural lobe.
- FIG. 6. Case 2. Microphotograph of the center of the basophil adenoma shown in Fig. 7. The cells are small, closely packed and moderately rich in granules. Palisading around the blood vessels is rarely evident. There are no indications of active degeneration.

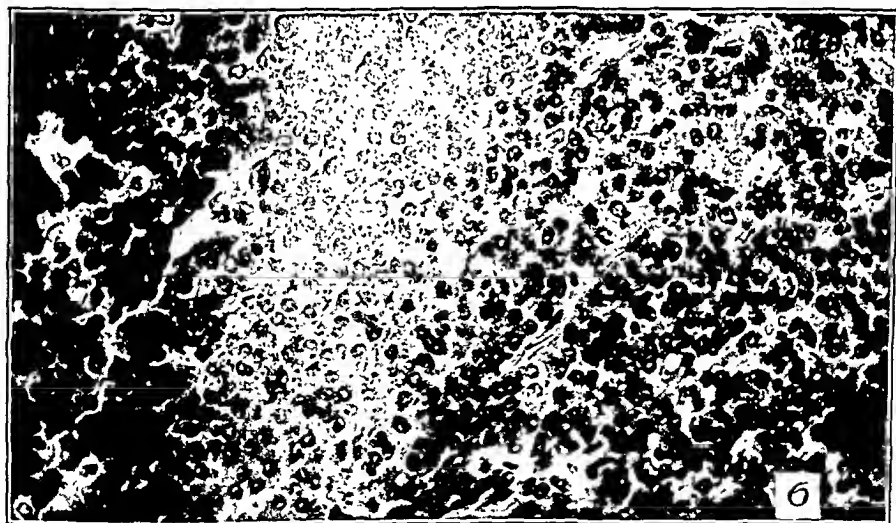
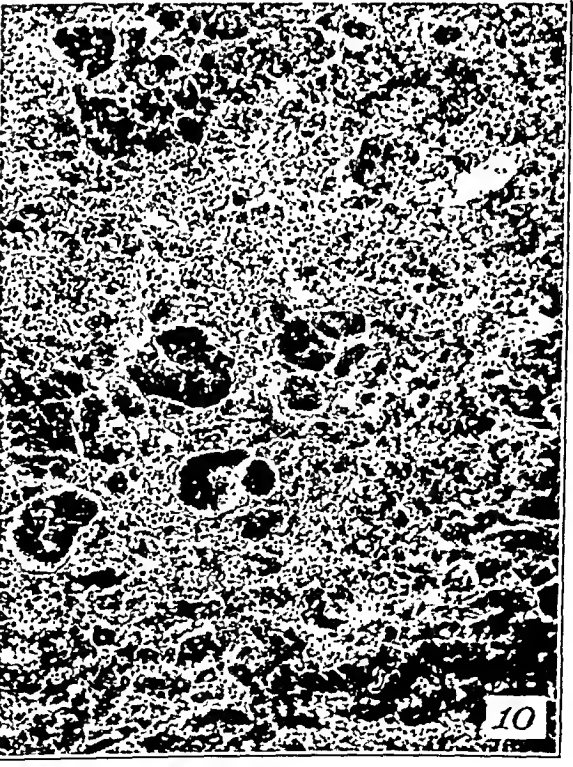
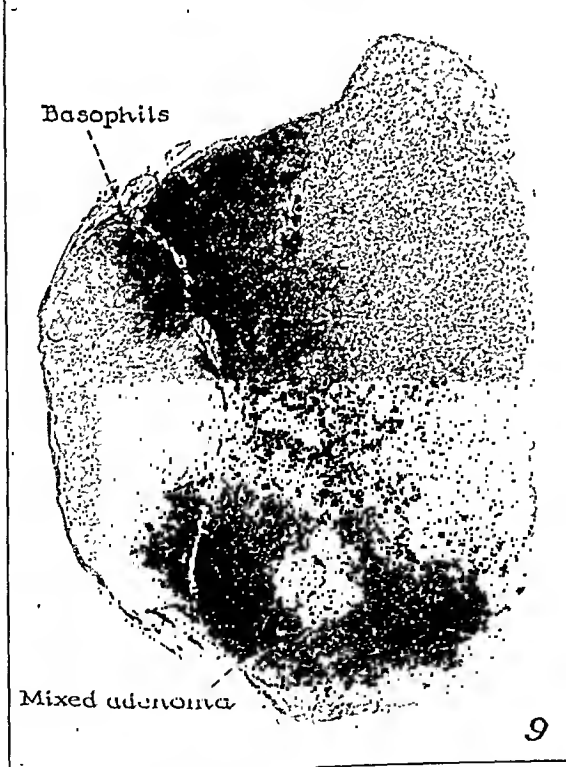
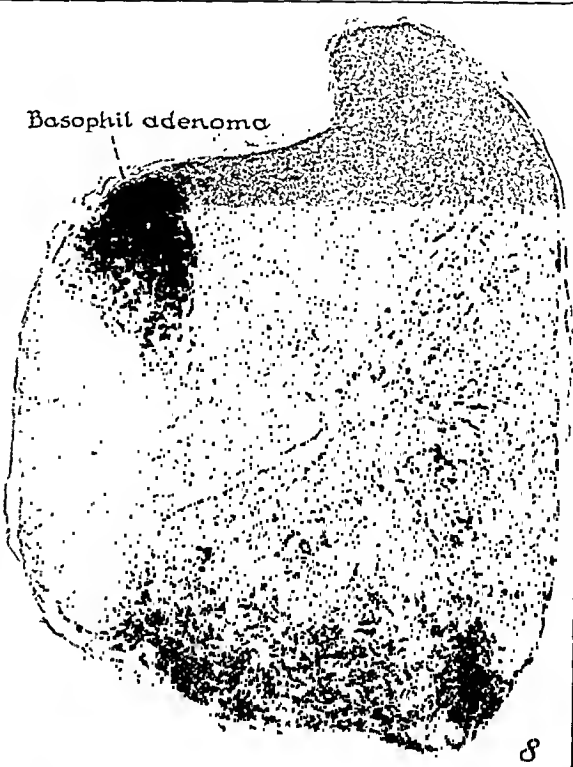
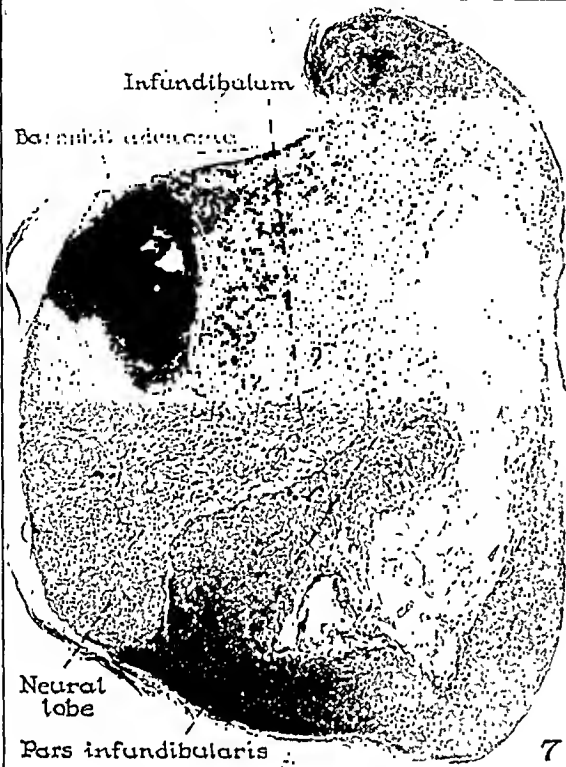


PLATE 61

- FIG. 7. Case 2. Microphotograph of a horizontal section through the upper rim of the hypophysis showing the location and general dimensions of the basophil adenoma. Part of the residual lumen and other evidences of the boundary between the anterior lobe and the pars intermedia are still present and indicate that the tumor is strictly within the posterior lobe.
- FIG. 8. Case 2. Microphotograph of a horizontal section about a millimeter lower than Fig. 7 showing the adenoma in the same relative position.
- FIG. 9. Case 2. Microphotograph of a horizontal section through the middle of the hypophysis and entirely below the adenoma where it has been replaced by a looser mass of basophilic cells derived from the pars intermedia, into which the tumor gradually merges. Diffuse basophilic invasion is present at this and all lower levels on the other side as well and to a lesser extent in the intervening region. In the anterior lobe is a small area having the appearance of a mixed adenoma containing many weakly basophilic cells.
- FIG. 10. Case 2. Microphotograph of a typical area of the anterior lobe of the hypophysis showing numerous small circumscribed masses of deeply basophilic cells. Normally such groups of cells are not rare but there is an excessive number in this specimen.





A STUDY OF THE SUBMAXILLARY GLAND VIRUS OF THE GUINEA PIG *

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INTRODUCTION

Intranuclear inclusions have been described in the salivary glands of the human infant and stillborn fetus,¹ in the monkey (*Cebus fatuellus*),² guinea pig,³ rat,⁴ mouse,⁵ hamster,⁵ and mole.⁶ The presence of a filterable virus in association with these inclusions has been demonstrated only in the rodents. The benign character of these infections, and the strict host specificity and organ selectivity of these viruses set them apart as a group of particular interest. The first of the submaxillary gland viruses to be discovered was described by Cole and Kuttner⁷ in the guinea pig in 1926, and since that date the results of a number of experimental studies of this virus have appeared in the literature. The present report deals with the submaxillary virus of guinea pigs with respect to spontaneous infections, the morphology and nature of the inclusion bodies, infectivity and virulence of the virus, and the immunological phenomena associated with infection.

METHODS AND MATERIALS

Virus emulsions for experimental infections were prepared from the submaxillary glands of guinea pigs supplied by local dealers, or from artificially infected stock animals. The salivary glands were removed under ether anesthesia from 3 or more animals and ground in a mortar with sterile sand. The pulp was taken up in approximately 1 cc. of physiological saline per gland. Gross particles were allowed to settle out or were thrown down by light centrifugation. The supernatant fluid was considered as a stock emulsion for purposes of dilution. Intracerebral injections of young guinea pigs and fetuses of the same species were made in 0.05 to 0.1 cc. doses. Fetal inoculations were made by the tech-

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† Mr. and Mrs. Frank G. Logan Research Fellow.

nique described by Woolpert,⁸ employing pregnant guinea pigs in the 4th and 5th weeks of gestation. Unless otherwise specified, all tissues examined histologically were fixed in Zenker's solution, embedded in paraffin and stained with eosin-methylene blue or eosin-azure.

SPONTANEOUS INFECTIONS

The cellular changes associated with the submaxillary gland virus were first observed by Jackson in 1920.³ The inclusions are found in the epithelial cells of the salivary ducts and less often in the renal epithelium. In the submaxillary gland the affected cells undergo a marked hypertrophy, a reaction that is characteristic of many virus infections and to which Unna applied the term "ballooning degeneration." Scott and Pruett,⁹ who made a detailed study of the volumetric changes in the cell, found that under the influence of the virus the cytoplasm may increase as much as 300 per cent and the nucleus as much as 400 per cent in volume.

Lesions may occur in the mucous accessory portion of the submaxillary gland, but are most often found in the serous part where they are commonly distributed in the smaller ducts near the periphery of the lobules. A single section may disclose one or more infected ducts with or without lymphocytic infiltrations varying from a cuff three or four cells deep to massive infiltrations which completely obscure the architecture of the duct and surrounding acini. Inclusion laden cells in the latter type of lesion may stain very poorly and be difficult to find. Such reactions are observed only in old adult guinea pigs and possibly indicate a slow resolution and elimination of infected foci.

In the kidney (Fig. 5), where the epithelium of the convoluted tubules becomes infected, the affected cells do not undergo the marked hypertrophy seen in the salivary gland. The intranuclear inclusions resemble those observed in the submaxillary gland and rarely acidophilic bodies are found in the cytoplasm of affected renal epithelium.

The incidence of spontaneous infection with the submaxillary gland virus in stock animals varies widely. Andrewes¹⁰ in England reported 32 per cent. In this country Jackson found inclusions in the glands of 54 per cent, and Cole and Kuttner in 84 per cent of the animals they examined. The incidence we have found in the

stocks of local dealers ranged from 7 to 74 per cent. Casual observation suggests that there is a relation between the sanitary conditions under which the animals are kept and the frequency with which positive glands are encountered. Histological evidence of infection in the glands of guinea pigs less than 1 month old is seldom found. However, that general absence of infection in young animals is not due to temporary resistance is shown by the fact that such animals are susceptible to intracerebral¹¹ and other routes of inoculation. Spontaneous infection may take place in the first few days of life, but 10 to 15 days must elapse before typical lesions can readily be demonstrated in the glands. Of 12 14 day-old guinea pigs examined, we found microscopic evidence of infection in 1. Cole and Kuttner found typical lesions in the glands of 3 of 43 guinea pigs, most of which were less than 1 month old.

Characteristic inclusions were present in the renal epithelium of 5 of 62 of our stock animals (8 per cent). The only report in the literature mentioning the incidence of spontaneous lesions in the kidney is that of Jackson, who reported the presence of inclusions in the renal epithelium of 12 of 44 adults (27 per cent).

The natural method of transmission of the virus has not been determined under experimental conditions. The presence of inclusions in organs such as the salivary glands and kidneys suggests that their secretions may be infectious. Using Berkefeld N filtrates of urine and saliva from known infected animals, we were unable to infect young guinea pigs by intracerebral inoculation. Two weeks after injection histological examination of the brain, salivary glands and kidneys revealed no characteristic lesions.

MORPHOLOGY AND NATURE OF THE INCLUSIONS

Preliminary attempts to study the structure of the intranuclear inclusions in frozen sections of the salivary glands were abandoned because of the fragile nature of the tissues and the scarcity of isolated cells suitable for observation. It was found that the meningeal exudate of intracerebrally inoculated animals offered greater possibilities for the study of inclusions in fresh unfixed cells. Preparations for examination were made by clipping small fragments of infected meninges from the ventral surface of the brain and placing them in a small drop of serum or saline on a

slide previously coated with a solution of Janus green and neutral red. It was observed that mononuclear cells containing inclusions take up extremely small amounts of the stains as compared with uninfected cells. The former cells are further characterized by their great size and distended nuclei (Fig. 1). Lying within the nuclear membrane and sometimes attached to it by barely perceptible filaments, the inclusion appears as a closely packed mass of refractile granules. As a rule only one such mass occupies the nucleus and the surrounding nucleoplasm is optically empty. As in the fixed and stained preparations, small clumps of chromatin can be seen in apposition to the nuclear membrane.

The granular character of the inclusion as seen in fresh mounts suggested that appropriate methods might disclose corpuscles of the sort found in certain other virus inclusions. Sections 5 to 7 μ thick were prepared from Zenker-fixed submaxillary glands of spontaneously infected guinea pigs. They were treated in the usual manner with xylol, alcohol, iodine and sodium thiosulphate, but were examined in the unstained condition in glycerin mounts. In such preparations the corpuscular nature of the inclusion is strikingly apparent on direct examination, though very difficult to photograph because of the absence of contrasting stains and the oblique lighting required for their visualization (Fig. 2). They appear as very small, round or oval bodies closely packed together and embedded in a less refractile matrix or ground substance. Because of the type of illumination employed it was not possible to measure them accurately.

Various dyes were used in attempts to stain the component parts of the inclusion. The best results were obtained by over-staining with Harris' hematoxylin and differentiating in an approximately half saturated solution of picric acid. Stained by this method the corpuscles appear discrete but considerably smaller than those seen in the unstained and undehydrated glycerin mounts (Fig. 3). In a few of the inclusions so stained these minute bodies are arranged in more or less concentric rings and resemble the elementary bodies reported in infectious ectromelia inclusions photographed by darkfield illumination and ultra-violet light.¹² On the basis of the evidence presented we propose that the nuclear inclusion is composed of units comparable to the elementary bodies found in some other virus infections.

Stains for fat, fibrin and amyloid substance showed that these substances are not present in the inclusions in amounts detectable by the usual methods. Silver impregnation methods added nothing noteworthy to our information on the structure of the inclusion.

We were able to examine tissues prepared by a modification of the Altmann technique.¹³ * This method involves fixation by rapid freezing in liquid air and desiccation *in vacuo*. The dry tissues are then quickly infiltrated and embedded in paraffin and sectioned in the usual manner. The method has the great advantage of yielding tissues that are not denatured by chemical fixatives and are therefore especially suitable for microchemical studies. Sections of infected submaxillary glands were thus prepared and stained with Feulgen's reagent for thymonucleic acid. Both the cytoplasmic and intranuclear inclusions react positively but with different intensity. The cytoplasmic bodies stain rather solidly in contrast with the intranuclear inclusions which often present a marked honeycombed appearance. This seems to be due to the affinity of the matrix material alone for the staining reagent. The elementary bodies retain none of the stain, thus giving the inclusion a vacuolated appearance (Fig. 4).

Other microchemical tests were made on sections prepared by the Altmann technique. Solubility tests (test interval of 5 minutes in each instance) were made with the following reagents: petroleum ether, xylol, ethyl alcohol, physiological saline solution, 0.5 per cent acetic acid, and normal HCl at room temperature and at 60° C. None of these reagents dissolved or materially altered the appearance of the inclusions. Pepsin and HCl digested the connective tissue but not the inclusions. A 2.5 per cent solution of Na₂CO₃ removed all chromophilic matter from the inclusions and all nuclei. The Millon test for protein was positive. While none of these microchemical tests has a high degree of specificity, collectively they indicate the protein nature of the matrix-substance of the inclusion bodies.

INFECTIVITY AND VIRULENCE

Experimental transmission of the virus is readily effected in young guinea pigs by subcutaneous, intraperitoneal, or intrave-

* Through the kindness of Dr. I. Gersh, then of the Department of Anatomy, University of Chicago.

nous injections of infective gland emulsions. After 10 to 15 days, inclusions are demonstrable in the salivary glands. As in spontaneous infections, the introduction of the virus by any of these routes is not followed by any marked symptoms of disease. However, when young uninfected guinea pigs are inoculated intracerebrally, they succumb to a specific meningitis. The time of survival or death of the animal and the incubation vary with the potency of the inoculum. In those animals that die acutely the incubation period is usually 5 to 8 days. The symptoms include ruffling of the haircoat, fever, tremors, relaxation of the urethral and anal sphincters, circus movements, and finally prostration. Death is generally preceded by a marked fall in temperature.

Histological examination of the brains of guinea pigs that have succumbed to intracerebral inoculation usually reveals a marked mononuclear meningitis, characterized by the presence of large numbers of intranuclear inclusions in the cells of the exudate. When the incubation lengthens to 15 to 18 days the symptoms are less marked, and histologically the lesions are more circumscribed and the inclusions are scant.

The effect of dilution on the infectivity of the virus is shown by titration in young guinea pigs. The results of such a titration are shown in Table I.

All of the animals that received the undiluted and the 1:10 dilution of the virus emulsion died. Sections of the brains of these 4 guinea pigs showed a typical meningitis with intranuclear inclusions in the exudate. The 2 that received the 1:100 dilution responded with a definite temperature rise and minor nervous symptoms, but both survived. The 3 animals that received the 1:1000 dilution manifested no significant symptoms of infection, and histological examination of the salivary glands of 2 of these animals after 7 weeks revealed no evidence of infection. Gland emulsions, when diluted 1:100 and 1:1000 produced fatal infections in guinea pig fetuses, whereas similar dilutions of the virus produced no more than a febrile reaction in 2 weeks old guinea pigs. The fetus, therefore, proves to be a more delicate test animal for the presence of the virus. This fact is doubtless due to the generalized character of the infection in the fetus. In it lesions containing typical inclusions develop in the meninges, liver, placenta and other organs, in contrast with the localized inflammatory response in the meninges of young postnatal animals.

TABLE I
Titration of Submaxillary Gland Virus Emulsion in Young Guinea Pigs

Animal No.	Inoculum		Days after inoculation							Result
	Dilution	Dose	1	2	3	4	5	6	7	
1	Undiluted	0.10	—	+	+	+	++	++		D 7
2	Undiluted	0.05	+	+++	+++	++	—			D 6
3	1:10	0.10	—	+++	+++	+++	+++			D 5
4	1:10	0.05	—	+++	+++	+++	++	++	+	D 11
5	1:100	0.05	—	+++	+++	+++	+++	+++	+++	S
6	1:100	0.05	—	+	++	+	++	++	++	S
7	1:1000	0.10	—	—	—	—	—	—	—	S
8	1:1000	0.05	—	—	—	+	+	+	+	S
9	1:1000	0.05	—	—	—	+	—	+	+	S

— = 39; + = 39.0-39.5; ++ = 39.6-40.0; +++ = 40.1-40.5; ++++ = 40.6-41.0 degrees C.
D = day of death
S = survival

IMMUNITY

Although no detailed study of the immunological phenomena associated with the submaxillary gland virus has been made in the present investigation, certain facts and observations have been brought to light which have a bearing on this question.

Cole and Kuttner reported that spontaneously infected animals were refractory to intracerebral inoculation, but Kuttner¹⁴ was later unable to satisfy herself as to the presence of neutralizing antibodies in the serum of infected adults. Andrewes¹⁰ subsequently showed that if the serum from immune animals was present in the tissue culture medium, inclusion bodies were not formed in susceptible cells.

Although the guinea pig placenta is of the hemochorial type (Grosser's classification) and thus is well suited to the passage of maternal antibody to the fetus, our fetal inoculation experiments have given no evidence that there is any protection conferred upon the fetuses of an immune mother. Likewise, the young of non-immune and immune mothers have been found equally susceptible to the virus. No increased resistance to infection occurred in newborn guinea pigs which were allowed to suckle immune mothers.¹⁵ It therefore appears that passive immunization *in utero* or *postpartum* is not an important factor in resistance.

Certain observations in the fetal inoculation experiments suggest that the duration of active immunity in the spontaneously infected adult is dependent on the existence of more or less active lesions in the salivary glands or kidneys. Following intracerebral injection of the fetus, there is frequently a localization of the virus in the placenta. This oftentimes calls forth a maternal inflammatory response which breaks down the fetal epithelium and allows the liberation of infected fetal macrophages into the maternal circulation. Under such circumstances and when only a few old and well infiltrated lesions were present in the salivary glands of the mother, evidence of very recent infection of the renal epithelium was found in some of the mothers (Fig. 6), whereas signs of reinfection were not found in the kidneys of the mothers when their salivary glands contained well developed but relatively uninfiltrated foci of infection with the submaxillary gland virus.

DISCUSSION

Unlike many of the naturally occurring viruses affecting laboratory animals, the submaxillary gland virus of guinea pigs appears to be thoroughly benign and of limited distribution in tissues. In a sense it bears a certain resemblance to the so-called "opportunists" among the normal bacterial flora of the body, in that it behaves as a pathogen only when fortuitously or artificially removed from its normal habitat and introduced into very vulnerable tissues such as the meninges or those of the fetus.

In this connection it is of interest to consider the distribution of intranuclear inclusions of unknown etiology in human salivary glands and other organs. The pertinent literature was summarized by Farber and Wolbach in 1932.¹ In the review of their findings, and those of other workers, intranuclear inclusions have been reported in the duct epithelium of the salivary glands in 35 of the 51 cases studied. All 35 were infants less than 2 years of age. Of the 17 cases in which inclusions were present in other viscera, 7 were either stillborn or premature and 1 was 2 days old. Except for 1 adult, none of the 17 was more than 2 months of age.

If it be presumed that the inclusions seen in these cases are of virus etiology, it follows that nearly half the individuals with visceral lesions acquired infection *in utero*. In addition to the striking morphological similarity of the inclusion bodies in both these human and guinea pig tissues, it is to be noted that the distribution and character of the lesions in the stillborn and premature group are remarkably like those we have described in the experimental infection of the fetus with the submaxillary gland virus of the guinea pig.¹¹ Moreover, the sites of the lesions in the 35 infants between 2 months and 2 years of age is in keeping with the localization of inclusions and virus in spontaneously infected guinea pigs.

Although many investigators have suspected the virus etiology of the inclusions in human salivary glands and other organs, to date there has been no report of successful isolation of a virus by animal inoculation. If such a virus exists and has properties similar to those of the salivary gland viruses of rodents, animal inoculation as a method of isolation is not likely to succeed because the viruses of guinea pigs, rats, mice and hamsters are

strictly species specific. Therefore, tissue culture of human tissues, such as those of the umbilical cord or of the stillborn, may offer a favorable medium for the isolation of the hypothetical virus.

SUMMARY

1. The characteristics of spontaneous infection with submaxillary gland virus of guinea pigs are described. The incidence in various local stocks of guinea pigs was found to be from 7 to 74 per cent. Inclusions were found in the renal epithelial cells of 8 per cent of adult animals examined.

2. Histological evidence is presented which suggests that the inclusion bodies associated with the guinea pig salivary gland virus are composed of elementary bodies similar to those known to occur in certain other virus diseases.

3. The relative infectivity of the virus is greater for the fetus than for the postnatal guinea pig.

4. Natural passive immunization *in utero* or *per colostrum* is inadequate to protect fetuses or suckling guinea pigs against experimental infection. In spontaneously infected adults the quality or duration of active immunity may depend upon the presence of active lesions.

5. An analogy between the distribution of the inclusion bodies sometimes found in stillborn and premature human infants and in experimentally infected guinea pig fetuses is pointed out.

Note: I wish to express my appreciation to Dr. N. Paul Hudson for his advice and guidance throughout the course of this study, and to the donors of the Mr. and Mrs. Frank G. Logan Fellowship Fund.

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DESCRIPTION OF PLATE

PLATE 62

- FIG. 1. Fresh, unstained wet mount from the meningeal exudate. The intranuclear inclusion appears as a granular mass in the center of the greatly distended nucleus. $\times 1450$.
- FIG. 2. Unstained glycerin mount of Zenker-fixed submaxillary gland. The hypertrophied cells lining the duct contain intranuclear inclusions which appear to consist of corpuscles or elementary bodies of uniform size. $\times 450$.
- FIG. 3. Longitudinal section of duct in submaxillary gland stained with hematoxylin and picric acid. The arrow indicates an inclusion in which the elementary bodies are arranged in concentric rings. $\times 450$.
- FIG. 4. Feulgen stain of infected duct cells in the submaxillary gland. The cytoplasmic inclusions stain deeply while the intranuclear inclusion has a delicately honeycombed appearance and stains less intensely. Altmann fixation. $\times 1000$.
- FIG. 5. Desquamated epithelial cell in renal tubule showing an intranuclear inclusion. Hematoxylin-eosin stain. $\times 1000$.
- FIG. 6. Intranuclear inclusions in the renal epithelium of a guinea pig whose fetuses were infected *in utero*. Eosin-azure stain. $\times 1000$.



1



2



3



4



5



6

Markham

Submaxillary Gland Virus of Guinea Pig

THE BONE AND CARTILAGE LESIONS OF PROTRACTED MODERATE SCURVY *

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TERMINOLOGY

In our opinion a great many of the words and expressions relating to conditions produced by vitamin C deficiencies are not well adapted to their purpose and in some cases are even incorrect. To justify the terminology used in this paper we shall briefly comment on this matter.

In the first place, the word *deficiency* refers to a concept that is hard to grasp. With regard to a vitamin, a state of deficiency exists in a diet that contains either none of the vitamin or any amount that is less than an adequate ration. More than the adequate amount results in excess. Thus, less than adequacy is deficiency and more than adequacy is excess, and both of these (deficiency and excess) can vary in extent; for instance, we can say a slight deficiency, or a great excess. However, a deficiency or an excess cannot exist in part so the commonly used terms "partly deficient" and "partial deficiency" are, strictly speaking, as meaningless as would be "partial excess" or "partly excessive."

The inaccuracies in the terminology of the conditions produced by various degrees of vitamin C deficiency are of a more serious nature than the foregoing. The word *scurvy*, being adopted before the cause of the disease was known, was originally defined by a clinical picture; to this a pathological picture was added later. The classical picture of scurvy is typified by the cases formerly arising on sailing vessels taking long voyages and in the first winter settlements of North America. This classical picture of the disease resulted from a great deficiency of the vitamin. But this is not the condition that interests us to-day, because it is seldom present. There is, however, a great deal of evidence¹⁻⁷ that indicates slight and moderate deficiencies are by no means uncommon. The condition resulting from these deficiencies (slight and mod-

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erate) has been called "subclinical scurvy," "the subscorbutic state," "latent scurvy," "chronic scurvy," and so on. Our reasons for objecting to the use of these terms are as follows:

In our opinion there is no need to restrict the name scurvy to the classical picture of the disease (hemorrhages, loosened teeth, aching muscles, prostration, and so on) which is the result of a great deficiency. Now that the cause of the disease is known, we think the fundamental definition should be a genetic one; we would prefer to say that scurvy is *the disease that results from a vitamin C deficiency*. Just as there are degrees of deficiency, so the resulting disease varies in degrees of severity. We suggest that the condition produced by a great deficiency be termed *severe* scurvy, and that those conditions produced by lesser deficiencies be termed *moderate* and *mild* scurvy. The terms mentioned above which are in use at present for these conditions are not adapted to their purpose. For instance "subclinical scurvy" is a misleading term because it implies that no clinical manifestations of the condition are present, whereas it may be that they have not yet been identified; "chronic scurvy" brings with it the implication of length of time, which is not always the point in question; "latent scurvy" would be applied more accurately if it referred only to the time that elapses before any indication of the deficiency is manifest; and "subscorbutic state" is an ambiguous sort of term.

INTRODUCTION

The diaphyseal lesions of severe scurvy have been carefully studied in both man and the experimental animal by several investigators. Little attention, however, has been focussed on the epiphyseal lesions of severe scurvy or on any lesion of the long bones in the milder forms of the disease. As slight and moderate vitamin C deficiencies have been shown to be not uncommon¹⁻⁷ it seems desirable to learn what bone lesions they would cause. Furthermore there is the possibility that a study of the lesions from mild scurvy might throw a different light on the action of the vitamin than do studies of the severe condition. Finally it is of interest to investigate the epiphyseal lesions of any scorbutic condition; these lesions, so far as we know, have not yet been described. Consequently an experimental study embracing these

points was undertaken, guinea pigs being used as experimental animals.

MATERIAL AND METHODS

Approximately 60 guinea pigs of mixed breeds and of both sexes were used. Each litter of 3 or 4 animals was put in a cage by itself at the age of 1 month. One of the animals of each litter was used as a control and the experimental procedure was begun at this time on the remaining 2 or 3 animals. The basal diet, fed all the animals *ad lib.*, consisted of a mixture of equal bulks of bran and rolled oats, 56 per cent (by weight); skimmed milk powder, baked in an open pan at 100–110° C. for 12 hours with occasional stirring, 30 per cent; butter fat freed from curd, 10 per cent; dried brewer's yeast, 1.5 per cent; cod liver oil, 1 per cent; sodium chloride, 1 per cent; and ferrous lactate, 0.5 per cent. This basal diet was tested as to its scorbutogenic properties at frequent intervals throughout the experiment by feeding it alone to guinea pigs and ascertaining that they died of severe scurvy in approximately 1 month. The control animals received in addition to this basal diet, 1 cc. of orange juice per 100 gm. body weight, plus an additional 1 cc. as a margin of safety; this is the minimum adequate ration proposed by Dann and Cowgill.⁸ The experimental animals received in addition to the basal diet one of three different rations of orange juice, 0.5 cc., 0.75 cc. or 1 cc. per day. A ration once adopted was adhered to until the animal died or was killed.

The bones from one side of each animal were prepared for gross examination by drying them. The bones from the other side were prepared for microscopic study. For the latter purpose blocks that contained a joint were usually selected, in order that epiphyseal lesions might be studied. The material was fixed in Allen's fluid, decalcified in this fluid or in 5 per cent nitric acid, sectioned by the paraffin method and stained with hematoxylin and eosin.

OBSERVATIONS

Observations were made on five groups of animals: controls; those receiving no vitamin C; and three groups, each of which was subjected to a different degree of deficiency. We shall not describe the appearance of normal bones or of bones encountered in severe

scurvy, except for purposes of comparison. Furthermore, the three degrees of deficiency resulted in lesions which also varied only in degree. Therefore we shall not describe all three, but confine ourselves to the typical lesions encountered in the various parts of long bones of an animal suffering from a protracted moderate deficiency. For purposes of simplicity we will further confine our observations chiefly to those lesions seen in *growing bones*. This point is important because growing bones suffer from different lesions than mature ones.

GROSS FINDINGS

The long bones of the animals which grew under a handicap of deficiency were only slightly shorter than those of the controls but they were much lighter, dried specimens weighing only about three-fourths of similarly treated control bones. Furthermore, the bones of the experimental animals were fragile, rough and discolored, and they frequently exhibited fractures of the shaft near the epiphyseal plate. Many of the articulating surfaces were somewhat flattened and not uncommonly invaginated for a very short distance into the epiphyses.

MICROSCOPIC FINDINGS IN THE DIAPHYSIS

It is difficult to grasp the character and significance of the diaphyseal lesion of moderate scurvy unless one knows the function of the disc of trabeculated bone which exists in a normal growing bone on the diaphyseal side of the epiphyseal plate. During growth, as can be seen in Figure 2, the tubular shaft of compact bone does not extend to the epiphyseal plate; it terminates under the plate in a zone of trabeculated bone which has not yet become compact. Unlike the compact bone, this honeycomb of trabeculated bone is not limited to the periphery of the bony structure but extends across the medullary cavity and caps it. This is necessary because, since cancellous bone is not so strong as compact, there has to be more of it to afford the same support. In this location of active growth, however, it is admirably adapted to its purpose because it develops very quickly and thus serves as a strong and extensive temporary scaffolding until the more laboriously constructed compact bone can be consolidated in its periphery. When the periphery at any level is sufficiently

strengthened the central cancellous framework is there rendered unnecessary and is dispensed with. It is with this temporary scaffolding that the scorbutic lesions of the diaphysis are concerned; progressively greater deficiencies in vitamin C produce correspondingly greater deficiencies in the cancellous scaffolding and number of osteoblasts in this location.

In protracted moderate scurvy the formation of this cancellous scaffolding is affected far more at the central portion of the cap than at the periphery. A glance at the more centrally located portion of the epiphyseal plate and at the tissue on its diaphyseal side gives the impression that longitudinal growth has ceased, because there is scarcely any indication of bone formation in this region (Fig. 1). However, a glance at the periphery of the cap serves to correct this impression because in this location there are some indications of cartilage being replaced by new bony trabeculae. Thus, the chief effect of the subminimal ration is to diminish the number and activity of the cells concerned in building this temporary bony scaffolding; as a result of this, the amount of scaffolding constructed is likewise diminished and what small amount is constructed tends to be limited to the periphery of the bone. This allows the bone to continue growing in length, but because the amount of temporary scaffolding is diminished, the bone becomes exceedingly weak on the diaphyseal side of the epiphyseal plate, where we commonly found fractures.

Other findings of interest present were: (1) That portion of the epiphyseal plate which caps the marrow cavity was for the most part thinner than usual although in a few areas it was thicker than normal. The columns of cartilage cells in it were less regular than usual. (2) That portion of the shaft which normally consists of compact bone was not as dense or as thick as is normal. (3) Examples of Gerustmark (fibrillar or framework marrow) were not as common as they are in severe experimental scurvy. Even in specimens that showed very meager bone formation, Gerustmark was not necessarily a prominent finding.

MICROSCOPIC FINDINGS IN THE EPIPHYSES

Articular cartilage presents an uncalcified zone towards the joint cavity and a calcified zone towards the layer of bone which underlies and supports it. This shell of cartilage and underlying

bone is normally supported by a scaffolding of cancellous bone. The uncalcified cartilage, the calcified cartilage and the supporting bone were all affected by protracted moderate scurvy. The supporting bone was diminished in amount and in some places there was none to support the cartilage (Figs. 3, 5 and 6). In some animals the zone of calcified cartilage encroached on the uncalcified so that a greater percentage of the articular cartilage was of the calcified variety. (This can be seen in Figure 3 where the calcified cartilage is deeper in color even than the underlying bone.) The uncalcified cartilage, in many sections, appeared thinner than normal.

The above stated fundamental changes were often accompanied by secondary changes. The weakened shell of cartilage and bone was often found distorted and fractured. Broken fragments of articular cartilage were found in masses of fibrous connective tissue which originated from the capsule. Capillaries were sometimes seen making their way into the uncalcified cartilage and these almost reached the surface in many instances. In some places where the cartilage had given way, the surface was covered with a layer or several layers of large cells with eccentrically located nuclei and basophilic cytoplasm. These cells resembled osteoblasts but were not associated with bone matrix (Fig. 6). The tips of the menisci in knee joints were often fibrinous in character; Figure 5 shows this, as well as some fibrinous exudate lying beneath the tip of the meniscus.

The connective tissue capsule of the joint, as well as the muscles associated with the joint, also showed lesions, a description of which is, however, outside the scope of this paper.

COMMENT

Character of the Bone and Cartilage Lesions of Scurvy

The term atrophy is commonly used in connection with the bones in scurvy. This does not necessarily mean that scurvy is a disease that causes bone resorption. For bone is one of those tissues like epidermis which is constantly being worn away and just as constantly replaced. Hence a bone can become atrophic, either through increased resorption or decreased restoration. It is important to decide which factor is involved in scurvy.

With this in mind, reference to growing scorbutic bones reveals

that the portion of the shaft laid down before the onset of the disease is affected much less than the tissue laid down after the onset of the disease. Thus the growing bone indicates that scurvy inhibits the formation of new bone rather than the speeding up of bone destruction. The chief lesions are located in the sites that are concerned with increasing the size of the bone, namely under the periosteum (where peripheral growth occurs), and in the metaphyses (where growth in length occurs). In investigating the effect of scurvy on growth we shall confine ourselves to its effect on that process which normally accounts for longitudinal growth. We shall do so because here the growth process and its disturbances are much more easily followed.

Our investigations into the effect of scurvy on longitudinal growth bring to light a paradox, namely that the bone continues to grow while very little new bone forms on the diaphyseal side of the epiphyseal plate. This calls for an explanation. The matter has been commented on by Park, Guild, Jackson and Bond⁹ in their extensive report concerning their X-ray and histological studies on the scorbutic bones of children. They believed, if we interpret them correctly, that bones continue to elongate in scurvy because the cartilaginous epiphyseal plate, on which longitudinal growth depends, continues to grow. They believed, moreover, that this cartilage was not replaced by bone but accumulated on the diaphyseal side of the plate as a honeycomb of heavily calcified cartilage, which they called the "lattice." This heavily calcified lattice showed up readily in X-rays and so provided an excellent means to help diagnose early scurvy in children.

Our conception of the sequence of events that occur in the metaphysis and that allow growth of bone to continue in scurvy is somewhat different from that of Park, *et al.* As stated, we generally found that the epiphyseal plate was thinner and we did not find accumulations of calcified cartilage at the diaphyseal side of the plate. Therefore, we do not think that an accumulation of calcified cartilage is a correct explanation for the continued growth of bone in scurvy. We had many sections (and Fig. 1 is a good example) that showed almost no accumulation of cartilage and almost no new bone formation at the center of the diaphyseal side of the plate, and we reached the conclusion that the central part of the plate becomes more or less dormant in scurvy. We

think that under these conditions its diaphyseal aspect would become heavily calcified and so show up in X-rays, thus accounting for the "lattice"; this "lattice" would further be thrown into sharp relief, as Park, *et al.*, point out, because there would be so little bone on its diaphyseal side. We think that longitudinal growth of bone continues under these conditions because the plate, almost dormant in its central regions, is carried along by growth activity in its periphery.

If the supplies of growth-energy for forming new bone, which are apparently inadequate in scurvy, were distributed equally in all the places where new bone normally develops, there would be so little for each part that longitudinal growth would be very slight. Instead of this, Nature gives the greater part of her restricted ration to the periphery of the metaphysis, and she cuts down at those places, *e.g.* the center of the plate, where new bone would give strength but where it is not essential to growth. The periphery of the metaphysis is the only place where this slight ration would maintain the growth of bone. Hence, just as a normal bone is a striking example of nature's architectural abilities when she has an abundance of material, so scorbutic bones are witnesses to the fact that nature is equally capable of making the best of a bad situation caused by lack of workmen and building materials. The effect of scurvy on epiphyses has not, to our knowledge, been described. In general the same effects are found here as in the diaphysis; there is a marked diminution in the amount of newly formed bone and a slight interference with the normal growth of cartilage.

Growth versus Maintenance Lesions

The next point we wish to discuss is whether or not our experiments give any clue as to the effects of long continued mild vitamin C deficiencies on the cartilage and bones of an adult. It must be realized that there are no criteria at present available by which the pathologist can identify the effects of deficiencies in such cases — that is, in the bones and joints of adults who were subjected to a prolonged slight deficiency after they had attained full growth. Our results indicate that bone and cartilage formation are diminished by any scorbutic condition. This deficiency manifests itself in growing bones at the sites where the growth occurs.

But in adult bone growth is over and the only new bone and cartilage that forms in them is for the purpose of replacing breakdown. It is well known that the structure of bone is always changing; old Haversian systems constantly break down and are replaced by new; and Elliott,¹⁰ in this laboratory, has recently shown that adult articular cartilage also gives evidence both of wear and of growth to replace wear. Consequently, in the adult we would expect to find the effects of long continued mild scurvy manifesting themselves not in special sites of growth, because there are none, but by general poor maintenance of the skeleton and articular cartilages caused by failure to replace breakdown. Curiously enough similar findings are characteristic of hypertrophic osteoarthritis. The bones in this condition become light and fragile. The term hypertrophic (referring to the presence of osteophytes) gives a false impression of increased bone growth; in reality there is far less bone in the skeleton than is usual. Having seen the way that nature uses her limited supplies of new bone in longitudinal growth, one would not be surprised to find that osteophytes are simply another example of nature's using her limited resources to best advantage. Osteophytes may be an attempt to prevent movement and thus protect both the cartilages and the bones. Others have already suggested a possible association between scurvy and rheumatic fever: we suggest furthermore the possibility of protracted mild scurvy (or a metabolic condition similar to that found in mild scurvy) being a factor in the defective maintenance of articular cartilages and bones so characteristic of osteoarthritis.

The Action of Vitamin C

In his recent review, King¹¹ states that "the two specific roles for vitamin C in animal tissues which appear to be clearly established are (a) respiratory function in serving as a hydrogen transport agent between unidentified metabolytes and other carriers of molecular oxygen, by way of two or more oxydase systems and (b) regulation of the colloidal condition of intercellular substances as shown by Wolbach and associates." As our findings did not suggest that vitamin C was concerned in the regulation of the colloidal state of intercellular substances, and as a careful study of the literature impressed us that the evidence for this

theory is far from satisfactory, we cannot agree with King that his function (*b*) of the vitamin is by any means clearly established. As a decision on this point is of no small importance, we therefore propose to review briefly the evidence that purports to support this theory and to discuss it in the light of our own and other findings.

The theory as stated by Wolbach and Howe¹² reads, "We therefore advance the theory that the failure of cells to produce intercellular substances in scorbutus is due to the absence of an agent common to all supporting tissues which is responsible for setting or jelling of a liquid product." We will henceforth refer to this as the "jellation theory" so as to avoid unnecessary repetition.

So far as we have been able to determine, the evidence for this theory is of two sorts.

1. That in scurvy there is an accumulation of fluid substance in both the bones and the teeth. This fluid substance is regarded as a secretion product of osteoblasts and odontoblasts respectively (unset intercellular substance) and it remains fluid in scurvy because of a lack of the jellation factor.

2. That in the healing of scurvy, firm intercellular substances form with such rapidity in the bones and teeth that the process is thought to indicate the jellation of previously elaborated fluid intercellular substance, the inference being that there would not be time for such extensive formation of completely new intercellular substance.

The evidence for the jellation theory obtained from (1) scurvy and (2) the healing of scurvy will now be subjected to scrutiny.

I. Evidence for the Jellation Theory from Studies on Scurvy

A. In the Bones: In 1919 Aschoff and Koch¹³ observed that zones of normal active bone formation were replaced in scurvy by a marrow framework (Gerustmark) which contained little in the way of firm intercellular substance. They came to the conclusion that the primary defect in scurvy was a lack of a cement substance without which firm intercellular substance could not be made. In 1926 Wolbach and Howe studied the bones in scurvy and stated that they believed the loose texture of the Gerustmark to be caused by the presence of liquid intercellular substance. Thus Aschoff and Koch, and Wolbach and Howe visualize continued secretion on the part of

the osteoblasts in scurvy but a failure of their secretion to jell.

In contrast with this we found relatively few examples of Gerustmark in protracted moderate scurvy. At the same time, and in sites where there was no evidence of Gerustmark, we found great interference with bone formation. In these areas of scanty bone formation there was no evidence of the accumulation of a fluid intercellular substance (Fig. 1); on the contrary the picture indicated that there was a general depression of all the activities usually concerned in bone formation.

B. In the Teeth: From their studies, Wolbach and Howe thought they obtained evidence of unset jell formation in the teeth in scurvy. They pointed out that the pulp became separated from the dentin and they thought this to be caused by a secretion of fluid intercellular substance by the odontoblasts. We think it would be very difficult to rule out artefact as a cause of the pulp being separated from the dentin in a cross section of an incisor tooth. In support of our contention we point out that Höjer and Westin's¹⁴ paper contains illustrations of cross sections of normal incisors with the pulp separated from the dentin and of scorbutic teeth with the pulp adherent to the dentin.

2. Evidence for the Jellation Theory Obtained from Studies on Healing Scurvy

A. In the Bones: Wolbach and Howe stated that their theory as a whole was supported by the promptness and volume of matrix formation following the administration of antiscorbutics. They found that after 3 days' administration of orange juice to a scorbutic animal bone matrix was formed, and that after 6 days there was a definite architecture of bone trabeculae.

We do not think that the formation of matrix and trabeculae in the time stated proves the existence of previously elaborated unset jell because Ham¹⁵ has pointed out that, in the repair of fractures of normal animals, completely new bone matrix can be formed in 48 hours and bony trabeculae in 4 days.

B. In the Teeth: The speed with which new dentin appeared in the incisor tooth of a scorbutic animal given orange juice also made Wolbach and Howe think that the process was one of the setting of the previously elaborated unset jell which had caused the pulp to separate from the dentin. Fish and Harris¹⁶ have re-

cently shown that this new dentin (variously called osteodentin, calcific scar tissue, secondary dentin and pulp bone) which appears in the pulp after antiscorbutics are given a scorbutic animal is a *normal* component of the tooth at certain levels; they have furthermore shown that it is a product of the pulp, and that it forms in the pulp from pulp cells and not between the odontoblasts and the dentin. As this material is a normal component of the pulp at certain levels, they point out that the interpretation of cross sections of incisor teeth is a very difficult matter. It should be remembered that Wolbach and Howe could not study the same tooth microscopically before and after the administration of orange juice. To avoid the difficulties caused by studies of cross section, Fish and Harris made their studies on longitudinal sections of molar teeth (the incisors curve in two directions and cannot be sectioned longitudinally). Fish and Harris' illustrations of scorbutic teeth show no accumulations of fluid between the pulp and the dentin which could represent unset jell formed continuously during the scorbutic period; on the contrary their illustrations show clearly that the calcific scar tissue which forms after the administration of antiscorbutics develops in the pulp just as it does normally near the wearing surface or after any injury to the tooth. Thus in the light of their work there seems to be no support for Wolbach and Howe's contention that the "new dentin" which forms on the administration of antiscorbutics represents the jellation of previously elaborated, unset jell.

In the light of the foregoing we conclude that the evidence for the jellation theory is decidedly inadequate. We think the lesions of bone and cartilage can be explained by a theory that postulates vitamin C to be somehow concerned in the metabolic processes of cells. Such a theory was offered by Höjer¹⁷ who, after careful studies on the tissues in scurvy, postulated vitamin C to be necessary for the growth and activities of cells in general and specialized cells in particular. This theory seems to us to be in accord with what we would expect from what has been found out about the vitamin in the field of biochemistry.

SUMMARY AND CONCLUSIONS

Protracted moderate scurvy was produced in young guinea pigs by feeding them diets containing less than adequate amounts of

vitamin C. It was shown that longitudinal growth of bone continued under this regimen only because most of the limited amount of new tissue which formed was in the peripheral part of the epiphyseal plate and in the ring of diaphysis adjacent to the periphery of the plate. This resulted in a weak shaft in this location and an unsupported epiphyseal plate. The chief manifestation of scurvy in the epiphysis was found to be a diminution in the amount of the bone supporting the articular cartilage. It was pointed out that in the adult, when growth is over, the effects of scurvy would become apparent in sites where continual replacement of tissue occurs to compensate for wear and tear. It was suggested that certain features of osteoarthritis (the poorly maintained articular cartilages, the generalized diminution of the amount of bone in the skeleton and the osteophytes) would be not unlikely effects of a long continued moderate vitamin C deficiency in the adult. Lastly the theory which postulates that vitamin C controls the jelling of intercellular substances was discussed in the light of other and our findings, and no support was found for this theory.

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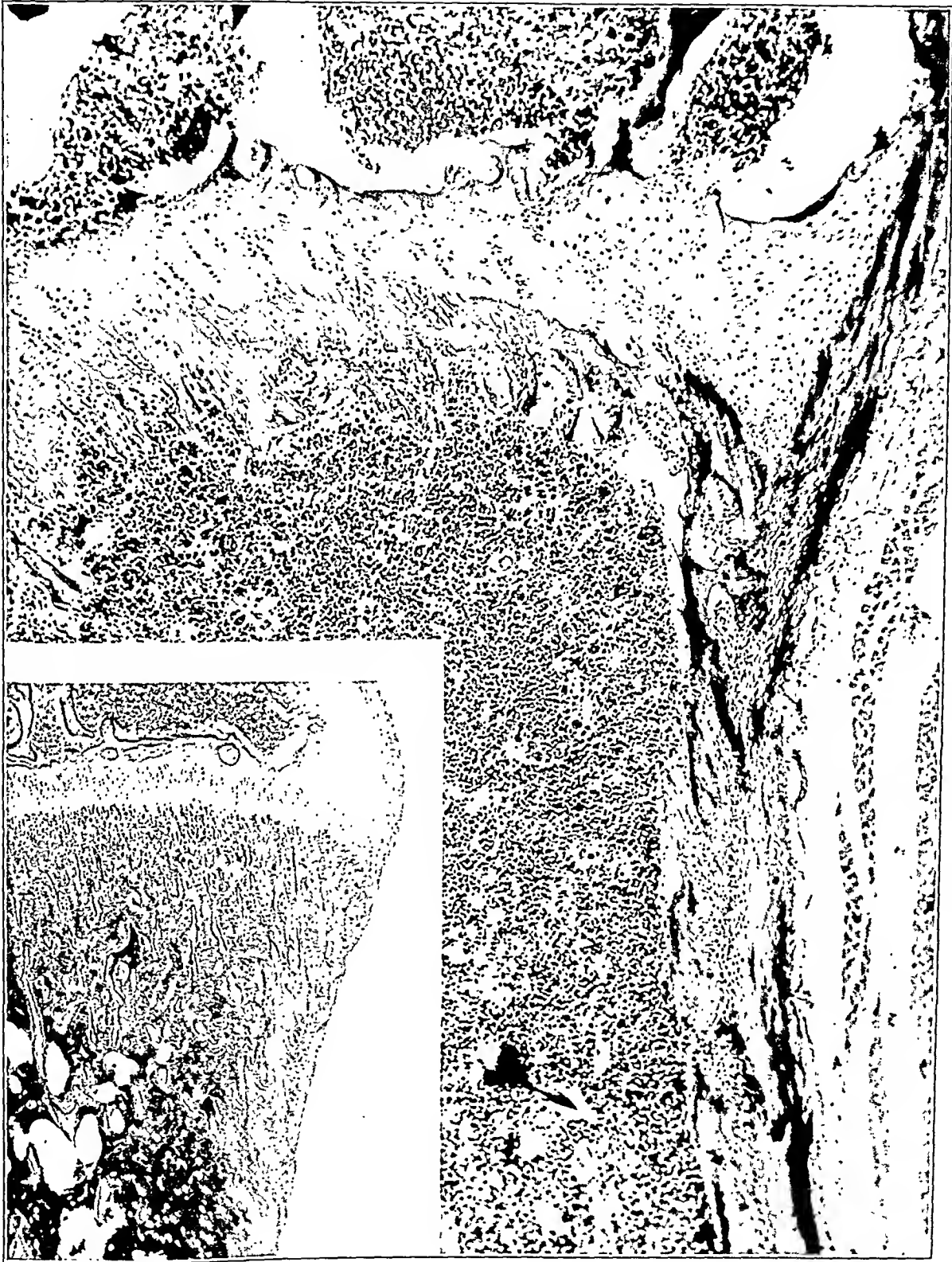
DESCRIPTION OF PLATES

PLATE 63

FIG. 1. Metaphysis of upper end of tibia from a guinea pig that received a normal diet for 1 month after birth, and thereafter 0.75 cc. of orange juice and no other vitamin C for 50 days, when it died. $\times 50$.

FIG. 2. Comparable section from a control animal of the same age. $\times 20$.

A comparison shows that the scorbutic bone lacks a normal trabecular framework in the center of the diaphyseal side of the epiphyseal plate; the marrow extends to the plate for there is absence not only of trabeculated bone, but also of calcified cartilage or semifluid Gerustmark in this location. At the periphery of the shaft enough new bone is formed to permit the shaft to increase in length. The shaft is very weak at this level, having almost no support of internal trabeculated bone.



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Ham and Elliott

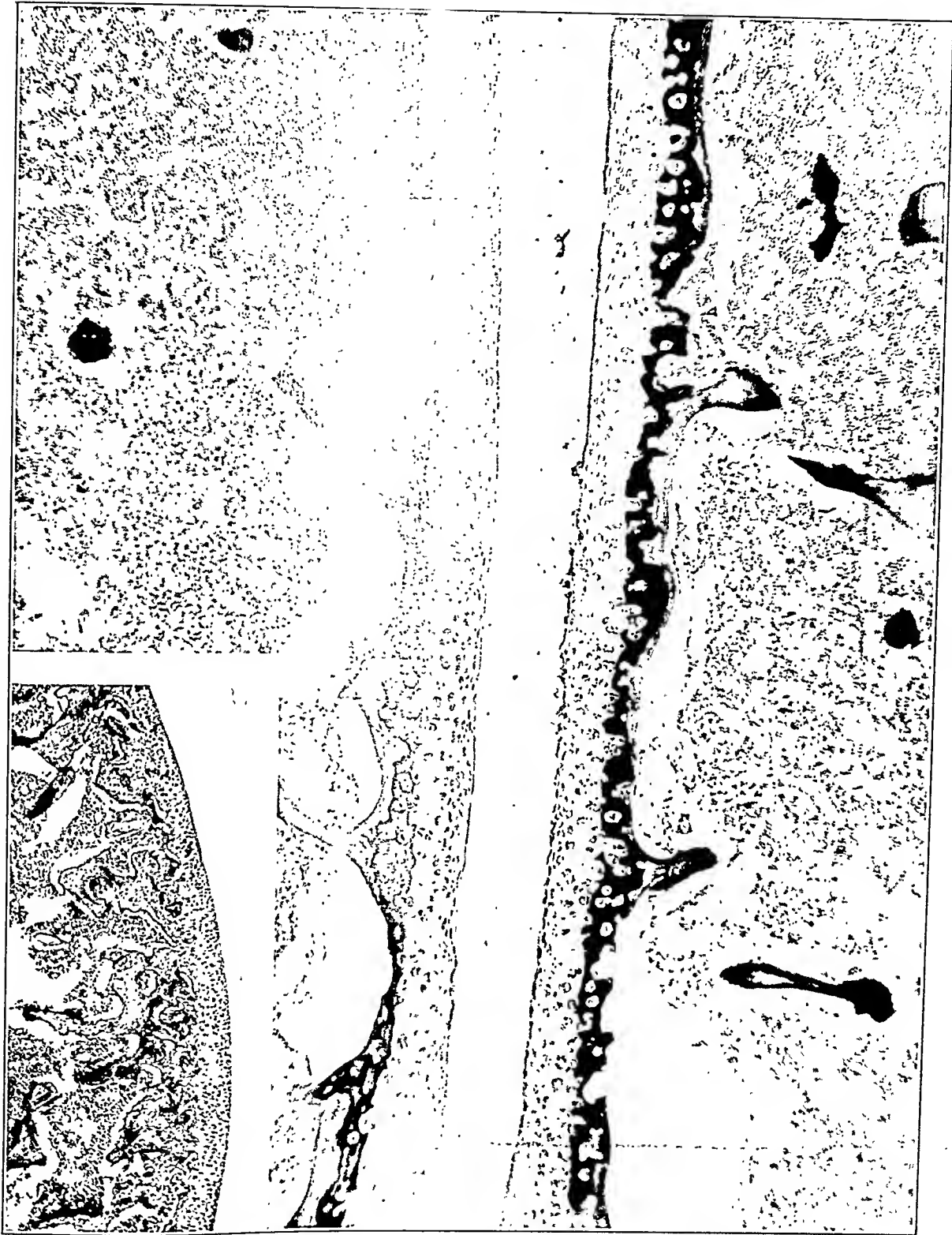
Bone and Cartilage Lesions of Scurvy

PLATE 64

FIG. 3. Knee joint of a guinea pig that received a normal diet for 1 month after birth, and thereafter 0.5 cc. of orange juice daily for 120 days, when it died. $\times 80$.

FIG. 4. Comparable section from a control animal of the same age.

A comparison shows that the scorbutic bone has a band of intensely staining calcified cartilage between the uncalcified cartilage and the supporting bone. The bone is lighter staining than the calcified cartilage, and is far less in amount than is normal. In a few places the cartilage is supported by no bone at all. The trabeculae of cancellous bone in the interior of the epiphysis are also fewer in number than is normal. This plate shows how protracted moderate scurvy diminishes the amount of bone that supports the articular cartilage.



4

Ham and Elliott

3

Bone and Cartilage Lesions of Scurvy

PLATE 65

FIG. 5. Section of the knee joint of a guinea pig that received a normal diet for 1 month after birth, and thereafter 0.5 cc. of orange juice daily for 83 days, when it died. $\times 80$.

The articular cartilage rests on almost no bony support, and in one location has given away (center of photograph). There is some fibrinous exudate directly above the fracture and the adjacent tip of the meniscus shows an inflammatory cell reaction. A few hyperchromatic nuclei at the site of fracture probably indicate a feeble attempt at repair.

FIG. 6. Section of articulating surface of the patella of a guinea pig that received a normal diet for 1 month after birth, and thereafter 0.5 cc. of orange juice daily for 120 days, when it died. $\times 80$.

This section shows an older fracture of the articular cartilage. There is almost no supporting bone and the calcified cartilage (very dark stain) is broken up. The area in which the cartilage has given way contains a proliferation of cells which resemble osteoblasts or tendon fibroblasts; these can be seen on the surface. Deeper (between the fragments of cartilage in the center of the photograph) there is some fibrous tissue.



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6

TUBERCULOSIS IN ALLERGIC AND DESENSITIZED GUINEA PIGS *

A STUDY OF HISTOLOGICAL CHANGES

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Tuberculosis, as it develops in normal and allergic guinea pigs, has been studied extensively, the literature on the subject showing rather general agreement that animals rendered allergic by infection with tubercle bacilli of low virulence are protected to a certain degree from subsequent infection with fully virulent microorganisms. There is less general agreement, however, on the question of whether or not the allergic state must necessarily accompany this partial immunity. Rich,¹ in a series of experiments with various diseases, has indicated that desensitization of allergic animals is possible without subsequent loss of immunity. As far as tuberculosis is concerned, Rich's contention is based largely on the work of Rothschild, Friedenwald and Bernstein.² Cummings and Delahant³ are the other workers in this country who have reported the treatment of guinea pigs with tuberculin in quantities sufficient to bring about complete desensitization. In both instances the desensitized guinea pigs were found to withstand subsequent virulent infection as well as the animals left in the allergic state. This contention is reinforced by the work of Yoshizawa⁴ who treated tuberculous guinea pigs with a form of precipitated tuberculin.

Our own studies^{5,6} indicate that the three above-mentioned papers do not present the complete picture of the relation between allergy and immunity because the experiments were terminated too soon. In the case of Rothschild and coworkers all of the experimental animals had died or had been sacrificed 65 days after the virulent infection. Cummings and Delahant sacrificed their animals at the end of 6 weeks. Yoshizawa's animals were killed at

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varying intervals, but none was allowed to live longer than 50 days. During the first 2 months after virulent infection our findings were essentially similar to those reported by the above workers. In the desensitized animals the liver and spleen remained relatively normal in gross appearance while the lung showed nothing but miliary foci, usually widely scattered. During the 3rd and 4th months, however, the desensitized animals died from extensive tuberculous pneumonia. In every instance in which the experiment was allowed to continue for 6 months or longer, we observed a 100 per cent mortality in the desensitized animals long before all of the allergic control pigs had died. Yet even in those desensitized animals whose lungs were completely hepatized by tuberculous pneumonia, the liver and spleen were usually devoid of tuberculosis.

The extensive pulmonary disease in our desensitized guinea pigs, along with the lack of tubercle in the other viscera, seems the more remarkable in view of the well known predilection for tuberculous infection exhibited by the guinea pig spleen. As Krause has said,⁷ "In guinea pigs . . . the spleen is undoubtedly the organ that is most prone to tubercle." And further, ". . . the guinea pig lung is highly resistant to infection. . . ." Our own experience with the development of tuberculosis in normal guinea pigs fully bears out Krause's statement. Nevertheless the fact remains that in the desensitized animals this "normal" picture is reversed, the lung becoming the seat of extensive disease while the liver and spleen are spared. This unusual phenomenon was deemed worthy of careful histological study.

As background for the comparison of tuberculous lesions in desensitized and control guinea pigs, we have had frequent occasion to refer to the above-mentioned paper of Krause, as well as to the studies of Willis.⁸ Most useful, however, for obtaining an idea of histological changes in all the viscera has been the exhaustive description of tuberculosis in guinea pigs by Pagel.⁹

EXPERIMENTAL MATERIAL

Desensitized animals of two different types were available for study: (1) allergic-desensitized — guinea pigs rendered allergic by infection with the R₁ strain of bacilli, desensitized by daily

injections of tuberculin* and then reinfected with virulent tubercle bacilli; and (2) normal-desensitized — normal pigs given 1 cc. of old tuberculin daily, then infected with virulent bacilli. In both types of animals desensitization was maintained by continuing the daily injections of tuberculin in amounts of 1 cc. or more, the amount never being greater than 2 cc. daily.

Along with the two types of desensitized animals, control groups of two types were employed: (1) normal controls — guinea pigs normal at the time of the virulent inoculation; and (2) allergic controls — guinea pigs allergic due to R_1 infection at the time of the virulent inoculation. In most of our experiments young albino guinea pigs were employed, the animals weighing between 250 and 300 gm. at the time of inoculation. In all, more than 500 pigs were used in our various experiments.

The microorganism used for the virulent inoculation was our "G" strain of human type tubercle bacillus. Relatively large doses were employed, the dose for different experimental series varying between 0.3 and 1 mg. The animals were inoculated subcutaneously in every instance. It is of interest that even with these wide variations in the inoculating dose, typical differences in disease picture appeared between the desensitized and the control animals.

Most of our animals were allowed to live until death occurred. However, in two experimental series animals were sacrificed at biweekly intervals for a period of 10 weeks in order to determine the number of tubercle bacilli in the spleen of each animal. As described in detail elsewhere⁶ this determination was made by culturing weighed amounts of each spleen according to the technic of Lurie.¹⁰ The results of these cultures indicated a fairly consistent difference in splenic content of bacilli in the three groups of animals that were employed. The allergic animals showed the smallest count, averaging less than 5000 bacilli per spleen. The allergic-desensitized animals stood between the allergic and the normal controls, averaging about 100,000 bacilli per spleen, while the normal controls averaged more than a million bacilli per spleen.

For our histological study tissues from lungs, liver, spleen and kidneys of animals in all of the series were fixed in formalin and

* The tuberculin used in these experiments was furnished by Parke, Davis and Company, Detroit, Michigan.

stained routinely with hematoxylin and eosin. When indicated, Ziehl-Neelsen's and van Gieson's stains were employed also.

OBSERVATIONS

Lungs

Control Animals: Typical tubercles, in the sense of circumscribed nodules of concentrically arranged epithelioid cells, are not found in the lungs of infected guinea pigs (see also Pagel). The pearly nodules, miliary in size, so readily seen in the gross invariably reveal themselves under the microscope as heterogeneous accumulations of epithelioid cells, lymphocytes, large mononuclears and a few polymorphonuclear leukocytes (Fig. 2). Desquamated alveolar lining cells filled with lipoid droplets play a part in completing the obliteration of all the air spaces in these small areas. During the later months of infection control animals of both the normal and the allergic groups may show areas of bronchopneumonia.

Desensitized Animals: Early in the 2nd month of infection miliary lesions appear in the lungs of the normal-desensitized guinea pigs. Similar lesions are found in the lungs of the allergic-desensitized pigs during the 3rd or 4th month. If the animals are maintained in the desensitized state until death occurs, the lungs, at autopsy, are distended, firm, and are dotted with large white areas of bronchopneumonia (Fig. 1). Histologically the lesion is seen to be largely interstitial in nature. In the less involved areas strands of solidified pulmonary tissue alternate with distended emphysematous alveoli (Fig. 3). In these solidified strands the interstitial tissue is infiltrated with polymorphonuclear leukocytes, lymphocytes and large mononuclear cells, and such alveoli as may be incorporated in the strand are obliterated, usually due to metaplasia of the alveolar epithelium to a cuboidal type. In the more extensively involved portions the interstitial tissue over large areas is infiltrated and most of the alveoli are obliterated in the manner just described. In addition, the terminal bronchioles and a few of the alveoli are found filled with dense plugs of exudate made up largely of granulocytes but containing also large mononuclear cells and desquamated epithelial cells (Figs. 4 and 5). Only rarely does one find definite caseation with destruction of the

connective tissue framework of the lung. Sections of the lung stained with carbol fuchsin reveal numerous acid-fast bacilli throughout the areas of interstitial pneumonia, and, in the bronchiolar plugs, the bacilli appear in great clumps as though here the microorganisms had found ideal conditions for growth (Figs. 6 and 7).

In addition to the histological sections we have examined numerous impression smears made directly from a cut surface of the fresh lung and stained for acid-fast bacilli. These smears reveal that some of the acid-fast microorganisms are within polymorphonuclear leukocytes (Fig. 8) while others are in cells of the mononuclear series. Occasionally, however, great clumps of the bacilli are found as though growing freely, without any suggestion of phagocytosis. Occasionally the smears contain non-acid-fast diplococci, but these are relatively few in number.

The large number of acid-fast bacilli in the desensitized lungs is the more striking when compared with smears from control pigs stained by Ziehl-Neelsen's method. Even though the lungs of the latter animals are dotted with tubercles, a tedious search of sections or smears may reveal no acid-fast microorganisms whatsoever, at most, only an occasional bacillus.

Liver

Control Animals: By the end of the 1st month the liver of the primarily infected control animal is almost invariably dotted with minute white foci. Under the microscope these miliary foci are seen as masses of epithelioid cells grouped mostly about the portal triad, though some are midzonal in distribution. An occasional giant cell may be seen and there is a slight infiltration with lymphocytes and granulocytes. With the lapse of time the periportal lesion continues to increase until, by the end of the 2nd month, the hepatic lobules are often completely outlined peripherally by tuberculous tissue.

Allergic control animals which have lived for several months show scarring of the liver, with connective tissue proliferation but no tubercle formation. The cirrhotic process is invariably of the portal type. Occasionally, in the larger areas of scarring, we have noted the proliferation of bile canaliculi.

Desensitized Animals: As already mentioned, the liver of the

desensitized guinea pig appears free from tubercles in gross. It is flabby in consistence and smaller in size than the liver of a comparable control animal (Fig. 1). The histological sections show atrophic parenchymatous cells and slight lymphocytic infiltration in the periportal region but no tubercles or tuberculous tissue. Occasionally the liver may show a small infarcted area.

Spleen

Control Animals: One week after infection of the normal control the malpighian bodies of the spleen show a slight polymorphonuclear cell infiltration. During the 2nd week epithelioid cells begin to fill the center of each malpighian body (Fig. 9) and, during the 2nd month, the normal lymphoid tissue becomes almost completely replaced by epithelioid cells. Spleens of the allergic controls exhibit a few conglomerate tubercles until several months have elapsed. Then they may show extensive fibrosis of the splenic pulp.

Desensitized Animals: The spleen of the desensitized guinea pig remains practically normal in size and exhibits no gross evidence of tubercle formation. By the 4th week after infection it shows, histologically, a fairly well marked band of tissue at the periphery of each malpighian body (Fig. 10). This band appears more eosinophilic than the surrounding splenic pulp and is demarcated rather sharply from the lymphoid cells of the malpighian body. When examined under high magnification the band is seen to be made up of reticulum cells in which there is a slight infiltration of polymorphonuclear leukocytes and a considerable accumulation of red blood cells. By the end of the 2nd month this band appears more dense and fibrous, a fairly definite perimalpighian fibrosis being the end result. A further change which occurs at these later intervals is thickening and fibrosis of the walls of the splenic sinusoids.

In the spleen, then, an initial perimalpighian lesion in the desensitized animal contrasts with the initial appearance of a central lesion in the malpighian bodies of the control animal. The lesions progress with time to the development of perimalpighian fibrosis in one case and to the almost complete replacement of lymphoid tissue with epithelioid cells in the other case. It should be mentioned again that, in spite of the absence of gross or microscopic

tubercles in the desensitized spleens, our bacteriological studies revealed the presence of large numbers of tubercle bacilli.

Kidney

Krause ⁷ has stated that "kidneys (of guinea pigs) practically never show gross tubercles"; and our own experience corroborates this statement. Nevertheless, in the present experiment the kidneys of all animals were subjected to careful scrutiny, not so much in the expectation of finding tubercles as to observe whether or not renal damage had occurred in the desensitized group from the excretion of tuberculin. We were not surprised, therefore, when definite renal lesions came to light in the desensitized animals, but we were not prepared for the finding of frequent lesions in the control kidneys as well.

No tubercles were found in the kidneys of either control or desensitized animals. However, in the majority of animals of both types, which lived as long as 2 months after infection and which were allowed to die from progressive tuberculosis, sections of the kidney revealed small cortical scars. In these scarred areas, involved glomeruli showed thickening and hyalinization of the glomerular capsule as well as of the glomerular capillaries. Frequently adhesions were found between tuft and capsule. The surrounding tubules were atrophic, the area being infiltrated with lymphocytes and large mononuclear cells (Fig. 11).

Lesions such as those just described were found with approximately equal frequency in the kidneys of desensitized and control pigs, the chief difference between the two groups appearing in the extent of tubular involvement. In many of the desensitized animals the convoluted tubules were extensively damaged.

Blood Vessels

In both control and desensitized guinea pigs that have lived for 8 weeks or more after virulent infection we have frequently noted vascular lesions. In the vicinity of the cutaneous ulcer caused by the primary inoculation the arterioles may exhibit an obliterating endarteritis (Fig. 12). More commonly, however, branches of the pulmonary artery and of the portal vein are involved. In animals that have been allowed to die from their disease the pulmonary arterioles are frequently found with the lumen completely oc-

cluded on account of contraction of the smooth muscle of the media. Hemorrhage in the surrounding pulmonary tissue indicates that this occlusion was an ante mortem rather than a postmortem occurrence. In the liver a similar marked contraction of the vessel wall is frequently found in branches of the portal vein.

DISCUSSION

Histological differences between the lesions in control and desensitized animals are much as one might expect to find them from the variations in the gross picture. The most pronounced difference is found in the lung where the "tubercle" of the control animal, a more or less circumscribed accumulation of cells, gives way in the desensitized animal to an interstitial pneumonia which comes to involve a major portion of the entire lung. An even more striking difference exists in the relative number of acid-fast organisms — very difficult to find in the lungs of the control pigs but innumerable in smears and sections of desensitized lungs.

It is interesting to speculate regarding the possible cause or causes of this great difference in bacillary content of the lungs. As has been pointed out elsewhere,⁶ the desensitized animals become markedly emaciated in comparison with the controls. They usually develop cutaneous ulcers which may persist for weeks. Furthermore, we have noted that after the injection of tuberculin each day the animals refuse to eat for several hours. They frequently leave portions of lettuce or other food untouched in the cage, with a resulting difference in the amount of vitamin intake as between the control and desensitized animals.

The inanition, emaciation, and ulcers of the skin all might be factors in bringing about the difference between the lungs of control and desensitized pigs. However, the one most important difference between the two types of animals is the difference in their allergic state, and this, we believe, may explain the observed changes. As Long¹¹ has so ably pointed out, tuberculosis, along with the other mycobacterial infections, is primarily a disease of cells of the monocytic type. Under ordinary conditions the guinea pig, following the development of allergy, seems enabled to exercise a certain amount of restraint on the growth of acid-fast microorganisms. But in the desensitized animal this restraining influence, at least in the lungs, seems to be lost completely. The

acid-fast bacilli appear there in great masses as though growing freely on excellent culture media.

A free proliferation of tubercle bacilli suggestive of that appearing in the desensitized lung has been observed in the guinea pig under entirely different conditions. It has been noted that tubercle bacilli, after intraperitoneal injection in normal guinea pigs, grow freely for a few days in (or on) the cells of the omentum.¹² It has been found further that this free growth period terminates in most of the animals 7 days after inoculation — at about the same time allergy begins to develop. Inasmuch as no free growth period was observed following the inoculation of timothy grass bacilli it was suggested that the capacity to grow freely for a period in guinea pig tissue is characteristic of virulent as distinguished from avirulent acid-fast bacilli and that protection from the overwhelming growth of virulent bacilli is a function of allergy.

It would seem that a comparison might legitimately be drawn between the above-mentioned work and the present study. In both instances we are dealing with non-allergic animals. Perhaps, in the lungs of the desensitized guinea pigs the tubercle bacilli, relieved of the restraining influence of allergy (and immunity), exhibit a phase of free and unrestrained growth comparable to the free growth in the omentum of the newly inoculated, non-allergic guinea pig.

SUMMARY

A study of histological sections from the organs of desensitized guinea pigs reveals extensive disease of the lungs with an overwhelming accumulation of acid-fast bacilli, while the liver and spleen show no definite tubercle formation.

Lesions in the kidneys and blood vessels of both desensitized and control animals are described.

It is suggested that the lack of allergy is responsible for the free and unrestrained growth of tubercle bacilli in the lung of the desensitized guinea pig.

DESCRIPTION OF PLATES

Figures 2-5 and 9-12 are from sections stained with hematoxylin and eosin. Figure 6 is from a section and Figures 7 and 8 are from impression smears stained with carbol fuchsin-methylene blue.

PLATE 66

FIG. 1. 1581 D. Lung, spleen and liver of *normal-desensitized* guinea pig 8 weeks after virulent infection. Note extensive involvement of lung while spleen and liver show no tubercles.

1581 N. Lung, spleen and liver of *control* guinea pig 8 weeks after virulent infection. The spleen is greatly enlarged and both spleen and liver show tubercles. Involvement of lung is less extensive than in 1581 D. Three-fourths natural size.

FIG. 2. "Tubercle" from lung 1581 N. of Fig. 1. $\times 90$.

FIG. 3. Strands of solidified pulmonary tissue from less involved portion of desensitized lung. $\times 150$.

PLATE 67

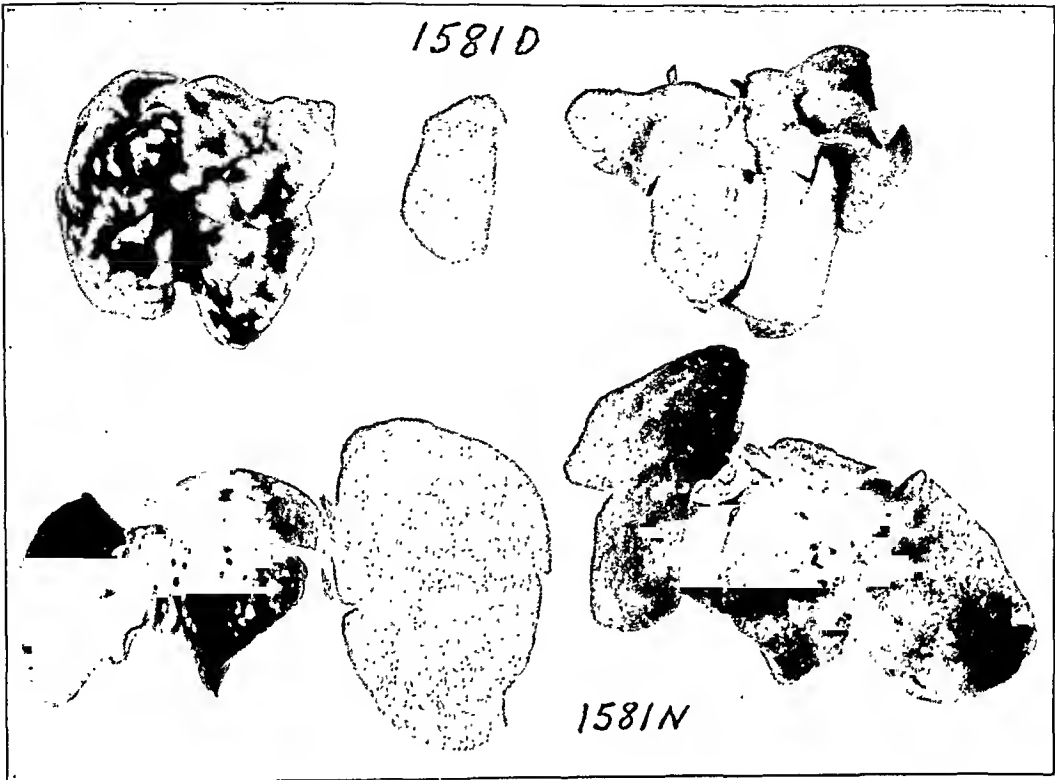
FIG. 4. Type of pneumonia that characterizes the lungs of desensitized animals dying from tuberculosis. The terminal bronchioles and certain of the alveoli are filled with dense plugs of exudate. This microphotograph is made from a section of lung 1581 D. $\times 75$.

FIG. 5. Enlargement of one of the bronchiolar plugs shown in Fig. 4. These plugs are made up of polymorphonuclear leukocytes, large mononuclear cells and desquamated epithelial cells along with great masses of tubercle bacilli. Note how the plug, during fixation, has pulled away from the wall of the bronchiole, leaving a wavy white space at each side of it. $\times 230$.

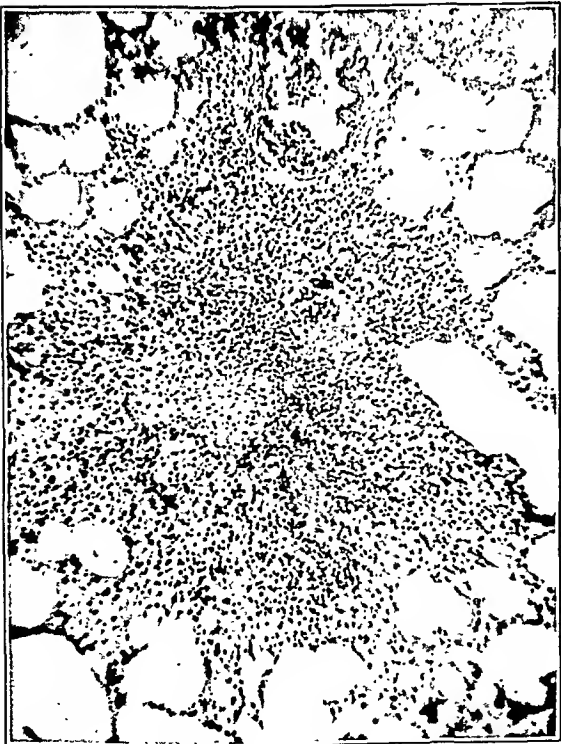
FIG. 6. One of the plugs of exudate within an alveolus. The photograph shows the acid-fast bacilli, only a few of which are in focus. Photograph taken with blue light. $\times 600$.

FIG. 7. Impression smear made from cut surface of lung 1581 D, showing the large clumps of acid-fast bacilli. Photograph taken with blue light. $\times 600$.

FIG. 8. Enlargement of a portion of the same smear. Each of the four clumps of bacilli illustrated is within a single polymorphonuclear leukocyte. Photograph taken with blue light. $\times 1500$.



I



2



3

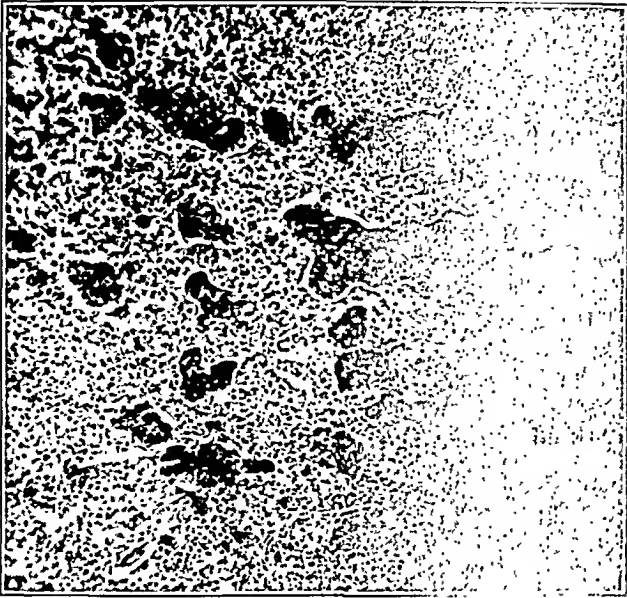
PLATE 68

FIG. 9. Malpighian body from spleen of control guinea pig 2 weeks after virulent inoculation. Note epithelioid cells in center of the malpighian body. $\times 120$.

FIG. 10. Two malpighian bodies from spleen of allergic-desensitized guinea pig 4 weeks after virulent inoculation. Note the light band (eosinophilic tissue) at the periphery of malpighian bodies. $\times 75$.

FIG. 11. Small renal scar in kidney of control guinea pig 8 weeks after virulent inoculation. $\times 230$.

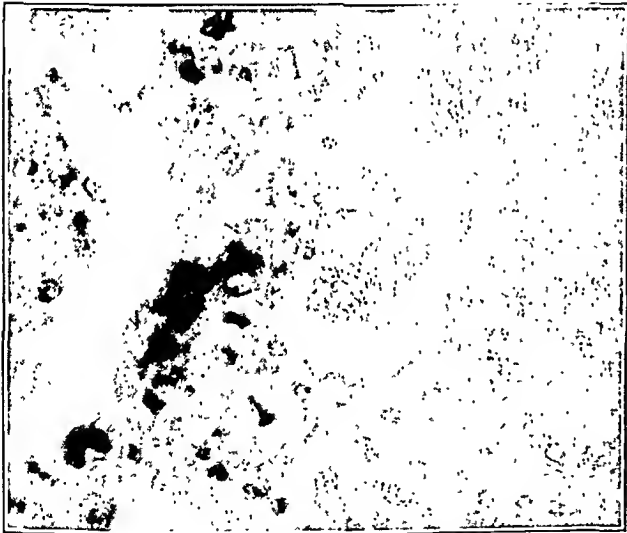
FIG. 12. Obliterating endarteritis in small artery found in vicinity of the cutaneous ulcer caused by primary inoculation with tubercle bacilli. $\times 350$.



4



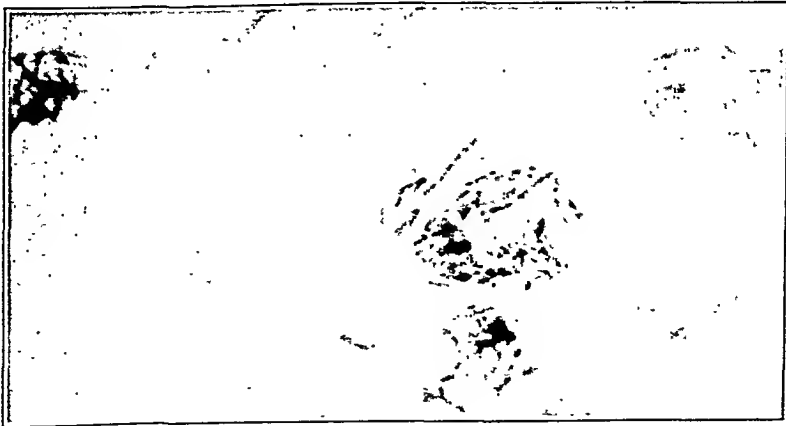
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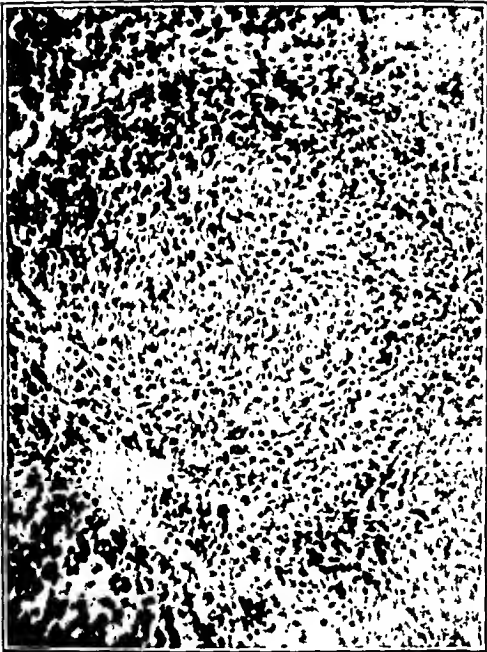
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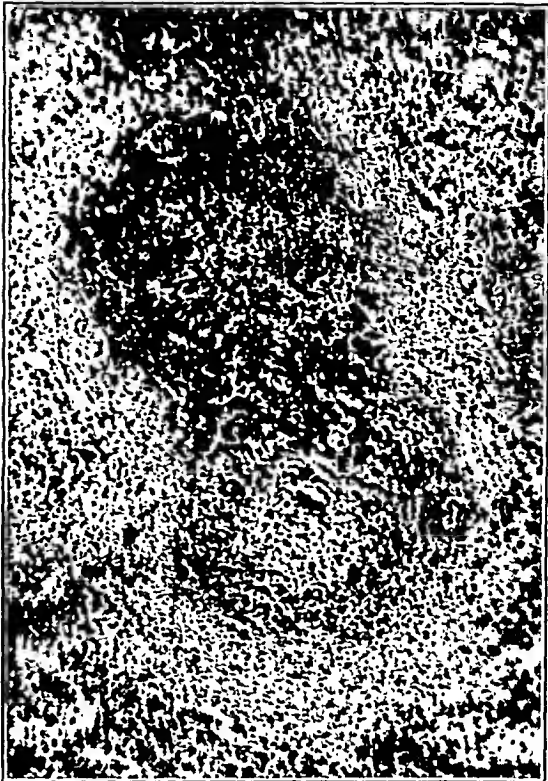
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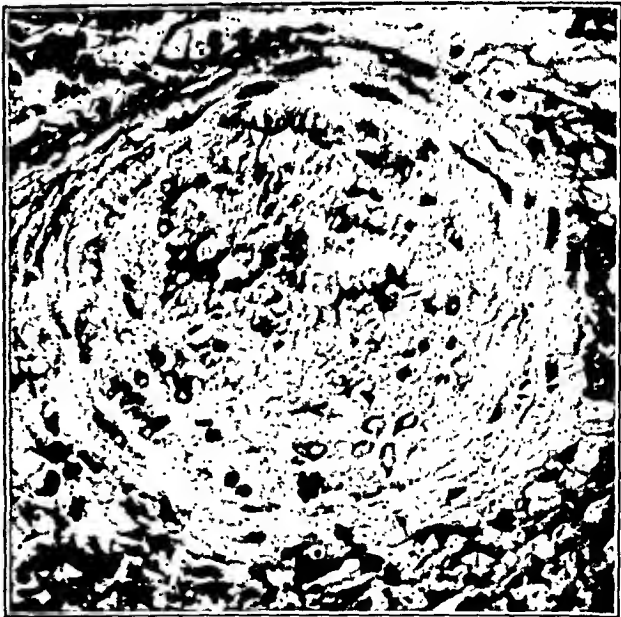
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10



11



12

Willis and Woodruff

Tuberculosis in Guinea Pigs

Saikowsky, in 1865,⁶ is the first reference I can find to studies in which fatty livers were produced in experimental animals. By feeding phosphorus to three rabbits he found the fat content of the liver increased from a normal of 5 to 6 per cent, to 8, 12.5 and 11 per cent, respectively. By this analysis it was shown that fat infiltration in the strict sense occurred. In 1883 Lebedeff⁷ showed again that the fat accumulation in the liver in phosphorus poisoning was the result of actual transport or migration into that organ.

In 1870 experiments by Ruge⁸ designed to show the effect of alcohol on dogs showed incidentally that diet influenced the results considerably; that alcohol-treated animals on a milk or meat diet only, accumulated fat in the liver up to 63 per cent of their weight. When bread was added to the diet fat did not increase to the same extent in the liver. Rosenfeld in 1902⁹ pointed this out later and established the fact that starvation is of primary importance in the development of fatty infiltration under experimental conditions. Statkewitsch in 1894¹⁰ had described fatty changes in the muscles and liver in fasting and wasting diseases, such as typhoid, and mentioned that of all glands the liver is the most affected. This was essentially a starvation effect. While there have been other similar observations in man and experimental animals, Mottram's¹¹ report in 1909 proved the same thing in another way. By starving guinea pigs, rabbits, dogs, pigeons and cats he found that fat accumulated with astonishing rapidity in the livers, this showing a marked increase in as short a time as 24 hours. That the fat came from the normal fat depots was shown by the change in the iodine number of the liver fat. This (iodine number) decreased progressively, approaching that of normal fat, as the absolute amount of fat in the liver increased.

With few exceptions the infiltration of fat into the liver may be considered pathological. Virchow quotes Kölliker's observations that fat accumulated in small amounts in the livers of suckling animals. Coope and Mottram¹² noted some increase of fat in the liver during pregnancy and lactation in rabbits. Neutral fat may be found in small amounts after a meal containing a great deal of fat, but it is probable that in most other instances where neutral fat may be demonstrated, grossly or microscopically, the condition is due to some interference with normal carbohydrate-fat catabolism. The large number of conditions in which fatty infiltration may

FATTY INFILTRATION OF THE LIVER AND THE DEVELOPMENT OF CIRRHOSIS IN DIABETES AND CHRONIC ALCOHOLISM *

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Direct evidence that alcohol can cause cirrhosis of the liver has been demanded before it is accepted as the primary etiological agent in so-called alcoholic cirrhosis, though everyone knows that the association of the two for centuries has been that of a cause and effect relationship. The mechanism by which the cirrhosis develops has not been understood, and the introduction and acceptance of the terms atrophic and Laennec's cirrhosis as including all types that are obviously not biliary in origin have allowed confusion to prevail. Mallory in 1911¹ pointed out the differences in anatomical types of cirrhosis but there is still too little effort on the part of pathologists and clinicians to distinguish these types. The writer pointed out in 1927² that little could be learned about alcoholic cirrhosis of the liver, or any other kind, until we learned to classify them as to origin, progress and end result. There is no reason why I should enter into the endless discussion as to whether alcohol can or cannot cause cirrhosis of the liver. I wish to describe in this paper how it may cause it by first producing a fatty liver.

One of the first direct observations on the finding of fatty livers in subjects known to have been excessive drinkers of spirits was recorded by Rokitansky in 1849.³ Previous to his report Louis in 1846⁴ had observed fatty livers in one-third of his cases of death from phthisis. Underlying this effect from two apparently widely different conditions there exists a very similar physiological process, as we shall see. Virchow⁵ regarded a fatty liver as pathological and raised the question of fatty infiltration versus fatty degeneration, which has been a subject for discussion ever since.

* The experimental work cited in this paper was supported by the Christine Breon Fund for Medical Research.

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that death from an otherwise lethal dose of alcohol could be prevented by oxygen inhalation.

Himwich *et al.*²⁸ concluded that the altered metabolism, which they found after alcohol administration (increase of lactic acid, increased carbon dioxide, change of pH to acid side, and increase of sugar in the blood) was due to an inhibitory effect on cellular respiration.* These effects are similar to those produced by ether, chloroform and carbon tetrachloride, and it is noteworthy that the experimental cirrhoses of the liver which most nearly approach alcoholic cirrhosis were produced by carbon tetrachloride (Lamson and Wing,²⁹ Bollman and Mann³⁰), which has a depressant action on carbohydrates similar to alcohol, but a much more drastic action on the liver; and by phosphorus (Mallory³¹), which causes a fatty infiltration, as well as degeneration, of the liver.

An example of how the withdrawal of dextrose from body metabolism may result in a fatty liver is found in the effects of phlorhizin poisoning. Rosenfeld⁹ showed that fat accumulated very rapidly in the liver (within 48 hours) when animals were treated with phlorhizin, and disappeared as rapidly. It was he also who pointed out that diet influenced the results of such experiments, as well as those with alcohol. I found that guinea pigs injected with phlorhizin did not develop fatty livers as long as they were fed a normal diet but did accumulate fat in the liver within 24 hours when starved for a day before treatment was begun.† Phlorhizin not only withholds sugar by causing rapid excretion, but actually withdraws glycogen from the liver and muscles so that again the proper breakdown of fat cannot take place.‡ The point I wish to make by this review is that fatty infiltration of the

* It is usually asserted because of the marked lowering of the respiratory quotient when alcohol is being oxidized that alcohol has "taken the place of carbohydrate" or has a "sparing action on sugar." Higgins (*loc. cit.*) says that probably 20 to 40 per cent of the total metabolism in alcoholism is due to alcohol. There is no evidence that alcohol can replace sugar and perform the same function as sugar in metabolism. The lowered respiratory quotient must mean simply that the demand for the oxidation of alcohol is imperative, and that metabolism is diverted for a short or for a long time from its normal carbohydrate-fat oxidation function to that of ridding the body of a poisonous agent.

† Unpublished experiments.

‡ The antagonism between glycogen and fat in the liver was pointed out by Rosenfeld (*loc. cit.*) and the statement has been repeated many times (see Lusk, G., *Science of Nutrition*, W. B. Saunders Company, Philadelphia, 1928, 310). This term is unfortunate as regards its chemical implications, but it does express the inverse relation between glycogen and fat in the liver in many conditions.

occur or be produced experimentally include the wasting diseases, tuberculosis and malignant tumors particularly, in which there may be a demonstrable lipidemia; a diet in which carbohydrates are decreased to a minimum or are absent (meat diet in an herbivorous animal, Pagès¹³); and even to some extent in subjects who have been dying slowly for several days and either not eating or not assimilating food. The histological picture in the liver when this has occurred is a later stage of that usually observed in such cases, which is simple glycogen depletion. Likewise such poisons as ether, chloroform, carbon tetrachloride and alcohol definitely interfere with carbohydrate metabolism, and so with the proper oxidation of fat (Quastel,¹⁴ Lattes,¹⁵ Peters and Van Slyke,¹⁶ and Goldschmidt, Ravdin and Lucké¹⁷). The moderate fat infiltration of the liver found in vitamin B₁ deficiency is probably due also to the influence this substance has on the proper oxidation of carbohydrates (Peters¹⁸).

That dietary insufficiency is in part responsible for fatty infiltration in alcoholism has been shown many times both clinically and experimentally. Among recent clinical observations that severe alcoholism and nutritional deficiency are coincident are those of Romano¹⁹ in which 79 per cent of 131 alcoholics had had an inadequate food intake; of Patek²⁰ who found a significant relation between nutritional deficiency and alcoholic cirrhosis of the liver which was benefited by a high vitamin diet; and of Goodhart and Jolliffe²¹ who found the same conditions and also described a case in which in addition to polyneuritis, "cirrhosis" of the liver was present. Observations reported here are simply repetitions of this not generally appreciated combination of pathogenic factors.

That alcohol alone will produce fatty infiltration of the liver was shown years ago by Ruge, and is asserted by Leathes and Raper.²² Bollman and Mann²³ found that it increased the rapidity and degree of infiltration in dogs fed a high fat diet. That alcohol produces its effect by interference with tissue oxidation is shown by Higgins²⁴ on the effect of alcohol on tissue respiration, and Peters and Van Slyke¹⁶ include alcohol among the poisons that produce a "histotoxic anoxia." Barcroft,²⁵ McFarland and Barach,²⁶ and van Wulfften Palthe²⁷ have commented on the anoxemia present in alcoholic intoxication, and the latter showed

but the lungs were congested and the heart weighed 580 gm. The aorta showed only a moderate amount of atheromatous degeneration with remarkably little calcification. However, the peripheral arteries showed advanced medial calcification.

The liver weighed 1720 gm. It was firm in consistence, had a granular irregular surface, and the cut surface was paler than usual. On microscopic examination fatty infiltration was marked and perilobular fibrosis prominent. The portal areas were irregularly thickened and showed some bile duct proliferation and scanty infiltration with lymphocytes. The picture was that of a typical portal and fatty cirrhosis (Fig. 1).

CASE 2. A male, 62 years of age, was known to have had diabetes for 6 years. This was fairly well controlled with 10 units of insulin daily. On occasions this had to be increased because of dietary indiscretions. There was no history of intemperance so far as alcohol is concerned. The liver was said to have been enlarged 8 months before admission to the University of California Hospital. The patient entered the hospital the day after he had vomited about 300 cc. of fresh blood. He collapsed after another hematemesis of about 1000 cc. An immediate transfusion of 500 cc. of whole blood failed to save his life.

At autopsy the prominent findings were in the liver and gastrointestinal tract. There was no ascites. The liver weighed 1705 gm. The surface was nodular and throughout the organ masses of liver tissue from 0.1 to 1 cm. in diameter were separated by fibrous tissue strands. The tissue was of a yellowish brown color. The stomach contained about a liter of bloody liquid while the lower part of the small intestine contained blackish tarry material. Numerous dilated veins were present in the mucosa of the lower part of the esophagus. No actual bleeding points could be found. Microscopically the liver showed a well advanced portal cirrhosis with very little fatty infiltration (Fig. 2).

I have included these cases here to emphasize two things particularly; how, in man, the decreased oxidation of liver fat associated with diabetes results in a chronic fatty infiltration, and how this prolonged infiltration may lead to a cirrhosis of the liver that is indistinguishable from alcoholic cirrhosis. If poisoning by alcohol is added to a preexisting diabetes, the physiological effect should be augmented, as the two conditions, alcoholism and diabetes, are synergistic chemical states.

liver nearly always depends on the fact that carbohydrate, for one reason or another, becomes unavailable for tissue oxidation.

FATTY INFILTRATION AND CIRRHOSIS IN DIABETES

Fatty enlargement of the liver in diabetes mellitis has been observed casually for many years although reports are few. Pepper in 1884³² mentioned fatty accumulation and congestion of the liver but attributed this in part to intemperance. Pflüger in 1905³³ observed that the liver contained more fat than normal in partially depancreatized dogs with diabetes. Umber 1914³⁴ makes a terse statement to the effect that the liver is generally enlarged in diabetes and that this may be due to hyperemia, fat infiltration or incipient cirrhosis. Hanssen in 1936³⁵ found enlargement of the liver in juvenile diabetes and states this must be due to fat. This could be reduced by proper diet and insulin treatment. Cruickshank in 1915³⁶ demonstrated that fat loss in feces could be diminished by feeding pancreas to depancreatized dogs, and since then the fatty infiltration of the liver associated with pancreatectomy and its partial control has been discussed by the Toronto group (Allan, Bowie, Macleod and Robinson 1924,³⁷ and others), and by Chaikoff.³⁸ By prolonging the lives of depancreatized dogs it was possible to watch these fatty livers develop into a genuine portal cirrhosis of the liver similar to that found in man in diabetes and in alcoholism (Chaikoff, Connor and Biskind³⁹).

I wish to report briefly 2 cases of cirrhosis associated with diabetes which came to autopsy.

CASE 1. A male, 77 years of age, had had diabetes for at least 17 years. It was diagnosed after he had developed an intractable ulcer on a toe. He had lost weight from 185 to 145 pounds. With insulin and diet he became well enough to dispense with insulin after several months. During the course of his illness he neglected his diet a number of times and drank some wine. Gangrene of both feet developed again, which progressed slowly, and it was 4 months after this that he came into the University of California Hospital. He had palpable arteriosclerosis with sloughing gangrene of the 1st, 2nd and 3rd toes of the left foot. The blood pressure was 120 systolic, 80 diastolic. He died 3 days after admission, following a period of coma with marked ketosis and a blood sugar of 328 mg. per cent.

At autopsy the peritoneal cavity contained small amounts of fluid in scattered fossae. There was no fluid in the pleural

may be bile tinged. Fat is frequently present in sufficient amount to grease the knife or the fingers. The consistence is variable but more likely to be firm and tense.

Microscopically these livers show a marked fatty infiltration estimated in some instances as over 60 per cent of the total weight. The fat occurs in typical large globules, and when present in moderate amounts is most often central in location, though this varies in different lobules and seems not to be important. Liver cells at the periphery of lobules may be flattened out, show early atrophy, or may have small amounts of hyaline cytoplasm. Glycogen is usually absent but some may be present when sudden death has occurred and where fat had not completely replaced it. An important finding in many livers is the reproduction of the liver lobules as lobules. In the human liver the lobule may be visualized by noting the spacing of the portal areas usually equidistant from the central efferent vein. From portal area to portal area there is no line of demarcation between adjoining lobules. These become apparent in the excessively fatty liver. Each lobule now becomes a unit and becomes distinctly separable from the other by adjacent rows of compact, squeezed atrophying cells. The superficial appearance again is of cirrhosis, but close examination reveals no fibrous tissue retaining wall as yet, and special connective tissue stains prove the absence of such tissue (Fig. 4).

In most of these livers there is more or less bile retention. This may appear occasionally as fairly large plugs in bile canaliculi, but is most often seen as very small dots of greenish inspissated bile. It may collect in fine curved lines, appearing as incomplete, greenish scaly capsules around fat droplets within a liver cell. This whole picture is that of biliary obstruction, but in no case has there been an extrahepatic block. The obstruction is obviously intrahepatic and is due to the intense and fairly rapid swelling of the parenchyma. This swelling is also responsible for the ascites which is frequently present if the patient has not died suddenly from acute liver insufficiency.

The clinical history and findings in this group differ only in minor details. All individuals had been drinking a great deal for the preceding several weeks or months and had neglected to eat. All had been chronic alcoholics for several years before this last severe episode, and some had been hospitalized before, suffering

THE LIVER IN CHRONIC ALCOHOLISM

In making the following case reports I have attempted to separate those livers in which there was definite cirrhosis from those in which no fibrosis was present, though all of them, except when sudden death occurred, were clinical cases of cirrhosis. This was not easy to do because the fatty liver grades so gradually into the fatty liver with cirrhosis and then into the cirrhosis without fatty infiltration that the dividing lines are by no means distinct. They are based on a study of 47 cases coming to autopsy in which anatomical diagnosis of cirrhotic or alcoholic liver, or both, had been made. Of these cases 3 proved to be toxic cirrhosis, 1 a hepatoma in which a preceding cirrhosis was obvious, and 1 a biliary cirrhosis. The study then includes 42 cases showing the various stages of the lesions to be described. Many others were reviewed but these 42 seemed to me to be conclusive. I have chosen only a few examples for detailed description and these will represent a composite picture. Naturally details were lacking in many clinical histories, particularly the personal details as to diet and the amount of alcohol consumed, so necessary to such a study as this. Also, it is necessary to review these cases backward, from autopsy to history, in order to separate the cases of fatty infiltration only from those of cirrhosis.

Fatty Infiltration of the Liver without Cirrhosis

The typical fatty liver as seen at autopsy in chronic alcoholism has been described. It seems necessary, however, to repeat this with emphasis on certain features that are important in producing the consequent changes leading to cirrhosis. In this series the weight of the liver varied from 1700 to 3600 gm. The enlarged liver has a tense capsule because of definite parenchymal swelling. If the swelling has been chronic or has occurred many times, the capsule is thickened and less transparent than normal. The surface may be coarsely granular or lobulated, even in the absence of cirrhosis. The enlargement and distention of lobules between unyielding interlobular septums which penetrate the liver from the capsule produce an irregular surface that may be accepted as meaning cirrhosis. This lobulation is especially marked during life when the liver is full of blood. The color is usually pale or yellowish red, depending on the amount of blood present. The lobules

may be bile tinged. Fat is frequently present in sufficient amount to grease the knife or the fingers. The consistence is variable but more likely to be firm and tense.

Microscopically these livers show a marked fatty infiltration estimated in some instances as over 60 per cent of the total weight. The fat occurs in typical large globules, and when present in moderate amounts is most often central in location, though this varies in different lobules and seems not to be important. Liver cells at the periphery of lobules may be flattened out, show early atrophy, or may have small amounts of hyaline cytoplasm. Glycogen is usually absent but some may be present when sudden death has occurred and where fat had not completely replaced it. An important finding in many livers is the reproduction of the liver lobules as lobules. In the human liver the lobule may be visualized by noting the spacing of the portal areas usually equidistant from the central efferent vein. From portal area to portal area there is no line of demarcation between adjoining lobules. These become apparent in the excessively fatty liver. Each lobule now becomes a unit and becomes distinctly separable from the other by adjacent rows of compact, squeezed atrophying cells. The superficial appearance again is of cirrhosis, but close examination reveals no fibrous tissue retaining wall as yet, and special connective tissue stains prove the absence of such tissue (Fig. 4).

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The clinical history and findings in this group differ only in minor details. All individuals had been drinking a great deal for the preceding several weeks or months and had neglected to eat. All had been chronic alcoholics for several years before this last severe episode, and some had been hospitalized before, suffering

from the same symptoms as were present in their last illness. A few examples follow:

CASE 3. An unemployed male, 49 years of age, had been intoxicated for 5 months, during which period his meals were very irregular and often missed. On entry to the hospital he was markedly wasted and had tremor of the arms and legs. He had been a cocaine addict but had not used the drug (so he said) for several years. He died a week after admission with fatty infiltration of the liver. Glucose and a high carbohydrate diet had been given as part of the treatment, which had probably reduced the fat content somewhat.

CASE 4. A female, 47 years of age, who had been a recluse and was known as a chronic alcoholic, increased her alcohol consumption. Three months before entry vomiting spells began, the abdomen became distended, and slight jaundice appeared. In the hospital the liver was found to be large, and ascites and slight jaundice were present. The liver weighed 1550 gm. at autopsy, was markedly infiltrated with fat and contained small plugs of retained bile, but there was almost no interlobular fibrosis.

CASE 5. A male, 37 years of age, died from a fall while drunk. His liver weighed 2800 gm., most of which seemed to be fat. Fat was also present in small vacuoles, resembling an actual fatty degeneration and appearing somewhat similar to the liver in phosphorus poisoning. The only past history known was that he had been a chronic alcoholic.

CASE 6. A male, 40 years of age, entered the hospital with jaundice, nausea and vomiting. He had been on a prolonged alcoholic diet with very little solid food. The jaundice was increasing and the liver enlarging when he was operated upon. A large, swollen nodular liver was seen. Finding no biliary obstruction, an omentopexy was done. A biopsy of the liver showed marked fatty infiltration, outlining of lobules and an increase of fine fibrous tissue connecting portal areas (Fig. 3). The patient recovered and remained entirely well 2 years after complete clinical recovery. It is probable that the change of diet had more to do with recovery than the operation.

These cases present samples of histories that become distressingly monotonous and do not differ qualitatively from those in which a varying degree of cirrhosis was found. The individuals who lived long enough after complete prostration to be studied presented clinical replicas of cirrhosis of the liver. Also, they showed at autopsy few or no other effects of alcoholism such as cerebral edema. Most died in a very few days, sometimes hours, after admission to the hospital. In some instances the liver con-

tinued to enlarge after all alcohol had been stopped, and the jaundice and ascites increased.

LeCount and Singer ⁴⁰ in 1926 described a similar series of cases. Others are found in French reports. A few citations will represent the surprisingly meager literature. In 1878 Bazy ⁴¹ reported a case of a female, 38 years old, who was said to have been intoxicated all the time for an unknown number of years. She entered the hospital with tremors of the hands and face and with a slight jaundice. The liver was large and continued to enlarge over a period of a week until it reached the umbilicus. Jaundice increased progressively. The liver weighed 3520 gm., was of a yellow ochre color and granular on the surface. The lobules were distinct and the capsule fibrosed. There was marked fatty infiltration with early portal cirrhosis. He called it acute yellow hypertrophy.

LeCount and Singer ⁴⁰ described the large fatty livers found in alcoholics who died rather unexpectedly without delirium tremens or the common findings of acute alcoholism. They noted the lack of glycogen in the liver. They wondered if "whiskey fits" could be due to hypoglycemia, discussed the availability of oxygen for burning both alcohol and fat, and questioned whether the lessened destruction of fat could not be due to a lowered supply of carbohydrate to metabolize fats. So far as cause of death was concerned, the condition of the liver amounted to a virtual extirpation of the organ. Friedewald ⁴² found that in 7 of 9 autopsied cases out of 50 acute alcoholic deaths enlarged fatty livers were present, and stated that most of the 50 were in a state of starvation.

That fat may infiltrate into the liver so rapidly and to such an extent that intrahepatic block and hepatic insufficiency ensue is shown by the occasional occurrence of the same phenomenon as recorded here in depancreatized dogs. They have died with jaundice and a swollen fatty liver, but without evidence that the diabetes, beyond being responsible for the fat, was very severe.

Allan, Bowie, Macleod and Robinson ³⁷ perhaps first described this phenomenon. Three depancreatized dogs died and jaundice and bile-stained fatty livers were present. There were some degenerative changes in liver cells around the central veins, marked fatty infiltration, and the bile canaliculi contained bile. Fisher ⁴³ described essentially the same findings in two dogs. In the course of experiments on depancreatized dogs it was found that this con-

from the same symptoms as were present in their last illness. A few examples follow:

CASE 3. An unemployed male, 49 years of age, had been intoxicated for 5 months, during which period his meals were very irregular and often missed. On entry to the hospital he was markedly wasted and had tremor of the arms and legs. He had been a cocaine addict but had not used the drug (so he said) for several years. He died a week after admission with fatty infiltration of the liver. Glucose and a high carbohydrate diet had been given as part of the treatment, which had probably reduced the fat content somewhat.

CASE 4. A female, 47 years of age, who had been a recluse and was known as a chronic alcoholic, increased her alcohol consumption. Three months before entry vomiting spells began, the abdomen became distended, and slight jaundice appeared. In the hospital the liver was found to be large, and ascites and slight jaundice were present. The liver weighed 1550 gm. at autopsy, was markedly infiltrated with fat and contained small plugs of retained bile, but there was almost no interlobular fibrosis.

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dition was precipitated when strict dieting and insulin dosage were not rigidly controlled.* Four such cases occurred in the large group of dogs used to produce the cirrhosis of the liver referred to above. These four dogs became emaciated, jaundiced, did not eat, and died in from 20 to 40 weeks after the pancreas had been removed. At autopsy the livers were markedly enlarged, filled with fat, and there was marked bile retention. There was no extra-hepatic block and nothing else to account for jaundice except intrahepatic swelling. Pictures of them could be substituted for those shown here from the livers in chronic alcoholism.

If I have somewhat belabored this phase of a subject, which should be common knowledge to clinicians and pathologists, it has been with the deliberate intent to emphasize that a pathological condition exists which is not cirrhosis, but because of the history of alcoholism, the ascites, moderate jaundice and frequent emaciation, the clinical condition of cirrhosis of the liver may be duplicated. A continuation of this condition or reproduction of the condition so frequently that in between times the liver has not had time to return to normal, will lead to the irreversible histological changes of cirrhosis.

Alcoholic Cirrhosis Following Prolonged Fatty Infiltration of the Liver

The clinical histories in most cases of definite cirrhosis, early or late, may, I think, be omitted. These individuals all have several common factors: they have consumed a great deal of alcohol during the last few years before death; they have been (when definite inquiry was made) on a very irregular diet, sometimes amounting to complete fasting, and nearly always low in carbohydrates; they have usually become wasted, or have lost considerable weight; and the age is usually above 45 years. I shall record the following case only because of the authentic history and the clear-cut sequence of events, both clinically and pathologically.

CASE 7. A female, 35 years old, who had been of temperate habits, began to drink heavily following a family catastrophe. At the end of 23 months she deliberately set out to drink herself to death and achieved this end in another 30 days. During the 2 years she had eaten very irregularly, and during the last month almost nothing.

* Experiments in collaboration with Dr. I. L. Chaikoff.

At autopsy the liver weighed 1800 gm., was of a yellow color, and had the characteristic lobulated surface of cirrhosis. On section the lobules were distinct, all of them larger than normal but irregular in size and shape.

Microscopically fatty infiltration was so extensive that nearly every cell contained a large or small globule. The sinusoids and central veins were collapsed, invisible, and a well defined fibrous tissue wall was growing around the lobules and sometimes through them (Fig. 6). Close examination revealed the changes that are so frequently found in this group of cases when the degeneration and fibrosis are rapidly progressing; changes that must be considered pathognomonic of developing fatty cirrhosis. These are: atrophy of liver cells at the periphery of lobules (Fig. 7); hyaline degeneration of swollen cells, many containing rounded or irregular masses of hyaline cytoplasm (Fig. 8); the development of thick reticulum fibrils around degenerating cells and along the course of sinusoidal walls (Fig. 9); and condensation of reticulum and further fibroblastic proliferation to form the well defined, perilobular connective tissue which is definitive.

Because most cases occur during the 5th and 6th decades, the statement is usually made that it requires many years for alcoholic cirrhosis to develop. This, as we have seen, is not true. The fact is that men do not begin to drink the astonishing amounts needed to cause cirrhosis until, as a rule, after the age of 45 years. The amount consumed previous to this period has been of no importance if it has been moderate. Varying the degree of alcohol poisoning, and variations in food consumption may prolong the process indefinitely. The maintenance of physiological metabolic equilibrium within safe limits may be possible even when comparatively large amounts of alcohol are taken habitually.

The Development of the Lesion of Cirrhosis in Fatty Livers

There seems to be no room for doubt but that long continued fatty infiltration of the liver is a mechanical factor of great importance in the development of a fibrous tissue-retaining wall around distended lobules. This had caused collapse of sinusoids throughout the liver. In addition to pressure on the peripheral cells, there is a definite reduction in circulation and consequent anoxemia. Slow atrophy of cells is the usual result. Other livers

dition was precipitated when strict dieting and insulin dosage were not rigidly controlled.* Four such cases occurred in the large group of dogs used to produce the cirrhosis of the liver referred to above. These four dogs became emaciated, jaundiced, did not eat, and died in from 20 to 40 weeks after the pancreas had been removed. At autopsy the livers were markedly enlarged, filled with fat, and there was marked bile retention. There was no extrahepatic block and nothing else to account for jaundice except intrahepatic swelling. Pictures of them could be substituted for those shown here from the livers in chronic alcoholism.

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* Experiments in collaboration with Dr. I. L. Chaikoff.

from the liver and cirrhosis began to be noticeable. Among eight dogs there were four with fatty infiltration and cirrhosis, and four with cirrhosis and little or no fat. Kaplan and Chaikoff ⁴⁴ have shown elsewhere that fat diminishes from a high of 40 to 50 per cent in the course of time in depancreatized dogs and the animals become emaciated.

Dible, ⁴⁵ and Dible and Libman ⁴⁶ have shown that the degree of fatty infiltration of the liver during fasting (mice, rats, rabbits, guinea pigs) depends on the amount of fat present in the body. When the fat depots are depleted fatty infiltration does not take place. One reason then for the disappearance of fat in fatty cirrhosis is exhaustion of the fat reserve of the body. Another and perhaps more important reason is that alcohol consumption becomes less and cautious dieting begins when the cirrhosis has developed to the extent that a gradual chronic passive congestion of the gastro-intestinal tract becomes manifest. With the resumption of a normal diet, particularly as carbohydrates are increased, fat rapidly disappears from the liver. It is to be expected then that in patients who have been hospitalized for some time the liver will become devoid of fat. Especially is this true latterly because of the empirical use of glucose solution in all cases in which a damaged liver is suspected. Frequently, then, all signs of fatty infiltration — cell atrophy, hyaline degeneration, and even active fibroblastic proliferation — have disappeared and we see the familiar quiescent, fibrotic, now somewhat shrunken, nodular liver of alcoholic cirrhosis.

SUMMARY AND CONCLUSIONS

Fatty infiltration of the liver occurs in those conditions where, because of lack of intake or absorption of food, fat is mobilized from the existing fat depots; and where, because of internal interference with the metabolism of fat due to anoxemia or tissue anoxia, the accumulated fat cannot be broken down for use. In the first instance it results from external starvation; in the second from what may be called internal or tissue starvation. In both instances normal carbohydrate-fat metabolism does not take place. Among the conditions in which this normal metabolism is altered or inhibited are the various diseases which, by their nature, are called wasting; the disease diabetes; and that following the intro-

undergo a hyaline degeneration, sometimes with clumps of hyaline cytoplasm appearing in cells, reproducing the picture described by Mallory years ago (Figs. 7 and 8). In more acute cases there may be an associated coagulative and fatty necrosis of cells, though this is not so common and not essential. This type of degeneration is seldom so severe as when found in chloroform, carbon tetrachloride and phosphorus poisoning. It is most commonly found in instances of acute death. The liver that develops outstanding cirrhosis must survive such episodes.

Connective tissue proliferation begins anywhere in the periphery of the lobules from the cells forming the walls of sinusoids. It is frequently more prominent around the portal areas but it does not necessarily grow out from them. There is usually a thickening of the sinusoidal wall as the first noticeable feature. The normally thin wall of scarcely recognizable collagen fibrils becomes swollen, forming homogeneous thicker lines of collagenous material, and a delicate reticulum can be seen to form around degenerating cells. The fibroblastic proliferation that follows is a familiar phenomenon and the fibrous strands thus formed connect collapsed empty capillaries (sinusoids) with one another, eventually joining with prolongations from periportal connective tissue. In the average slowly developing case these strands more or less clearly define the limits of a lobule, with an efferent vein in the center and the now united portal areas forming "fence-posts" at regular intervals. These strands, however, may join with others coming from any direction and so cut across lobules, forming the irregular lobulated pattern that has been so hard to explain. The process after this is simply the development of more and more connective tissue with subsequent condensation and some attempt at regeneration of bile ducts and liver lobules (Fig. 10).

The disappearance of fat from the liver in advanced cirrhosis is readily explained, although the explanation in each case may not always be the same, or a combination of factors may operate in the same case. In the experiments previously described concerning depancreatized dogs, fat accumulation in the liver remained for periods varying from 3.5 to 4.5 years. These diabetic dogs were receiving insulin and a diet that allowed them to live comfortably without marked metabolic imbalance. In animals allowed to live for several years, varying in each animal, fat gradually disappeared

the second form in which further proliferation of connective tissue produces the typical picture of portal cirrhosis as seen in chronic alcoholism. An unmistakable gradation of the one into the other is so manifest that the mechanism of the production of alcoholic cirrhosis seems to me to have been demonstrated. The absence of fat in many such livers at the end is explained by the exhaustion of body fat, by the discontinuance of alcohol consumption, and the resumption of a normal or a high carbohydrate diet.

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duction of poisons which inhibit proper tissue oxidation, the most common of which is alcohol. Others occur but are not dealt with here.

In the starvation accompanying progressive morbid states the condition is of little pathological importance, being in most cases terminal in nature. In diabetes the enlarged fatty liver may be influenced by insulin and by strict dieting, but also may persist for many years. In alcoholism a variety of factors operate to produce the same condition. Alcohol alone will cause some fatty infiltration, but as relative and sometimes absolute starvation is constantly associated with severe chronic alcoholism, the development of fatty infiltration of the liver most often depends on a combination of these two. The absence of vitamin B₁ in the diet may contribute to this also, but such deficiency is probably of minor importance.

Experiments recited here, and an analysis of existing recorded observations and experiments, indicate that a liver containing demonstrable neutral fat is in most cases pathological; that fat may pass into and out of the liver with astonishing rapidity; and that fat may be present in such amounts as to interfere seriously with both the metabolic and the mechanical functions of the organ. In 2 cases of diabetes this chronic fatty infiltration went on to portal cirrhosis. One of these cases was so severe that the patient died of hemorrhage from esophageal varices. The development of perilobular fibrosis seems to be the result of a combination of mechanical pressure, local tissue anoxia and general anoxemia, causing atrophy and degeneration of liver cells.

The alcoholic liver occurs in two forms, one of which is the precursor of the other. The first is the enlarged tense liver so swollen with fat that the distended surface lobules present the appearance of cirrhosis. This effect is further simulated by the intrahepatic block, which interferes with excretion of bile, and the transmission of portal blood. The clinical signs of jaundice and ascites are thus produced. Many such patients lapse into coma and die, and at autopsy the large liver is the only prominent finding. These show lobules distended with fat and some show, in addition, hyaline degeneration and atrophy of peripheral cells associated with early proliferation of fibroblasts. A series of cases presented here represents this group, and another series illustrates

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DESCRIPTION OF PLATES

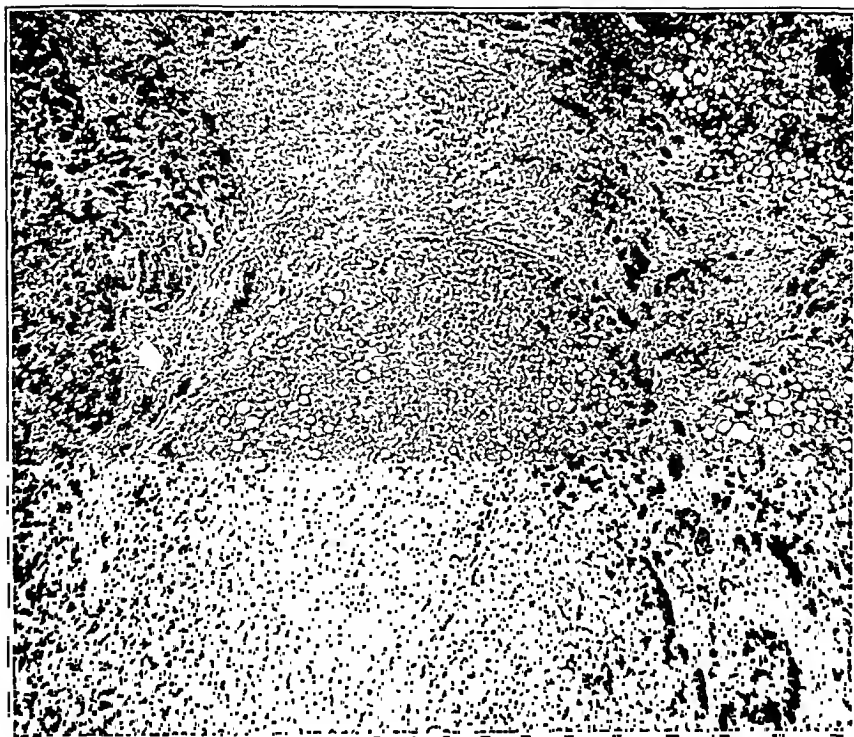
PLATE 69

- FIG. 1. Microphotograph of liver showing fatty infiltration, perilobular and intralobular fibrosis in a case (Case 1) of diabetes of 17 years duration. Death from diabetes. Hematoxylin-eosin stain. $\times 50$.
- FIG. 2. Microphotograph of liver (Case 2) showing perilobular fibrosis (portal cirrhosis) in a liver which was known to have been enlarged (presumably fatty) 8 months previously. Death from portal obstruction, esophageal hemorrhage. Hematoxylin-eosin stain. $\times 50$.

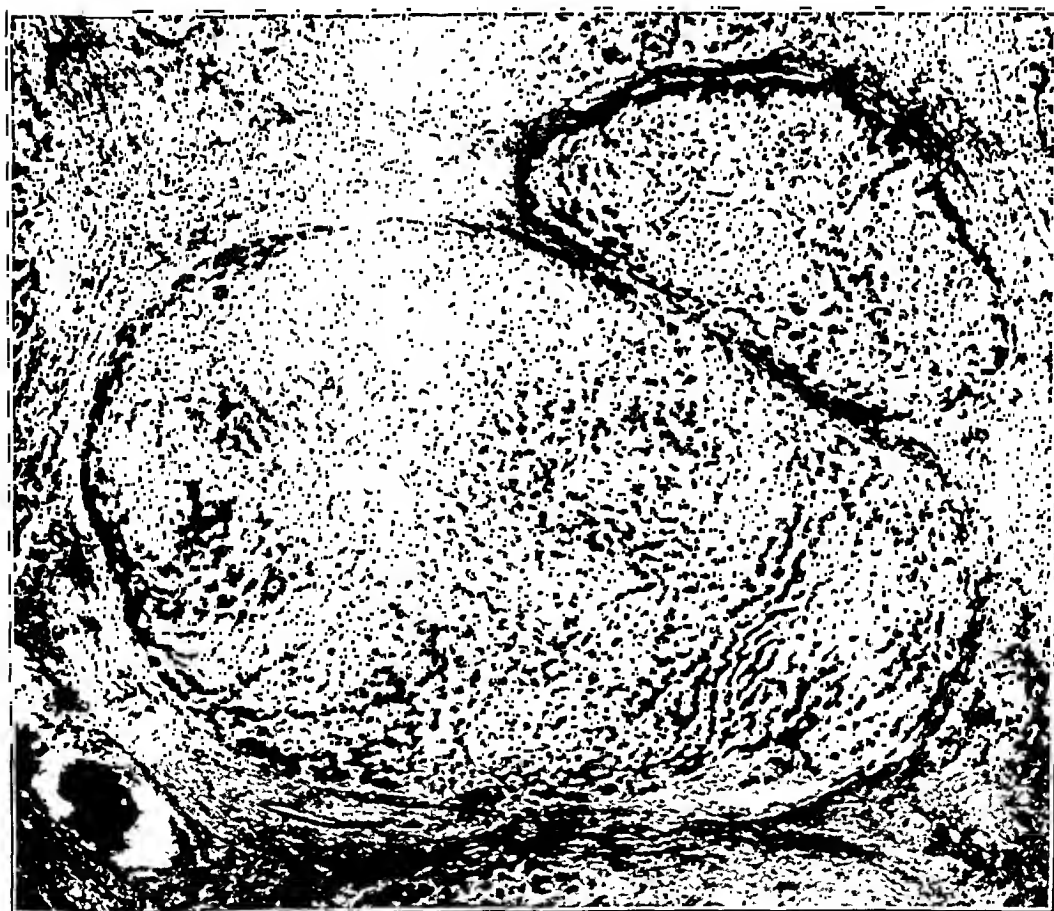
PLATE 70

FIG. 3. Microphotograph of liver. A biopsy specimen from a patient who recovered and has remained well. The effects of alcohol are seen in the marked fatty infiltration, peripheral cell degeneration and connective tissue proliferation. A borderline change which has returned to a normal physiological state so far as can be determined, but which, it seems obvious, could progress into the irreversible condition of cirrhosis. Mallory's aniline blue collagen stain. $\times 60$.

FIG. 4. Microphotograph of liver showing fatty infiltration (acute) from a male who died of alcoholism. The outlining of the lobule is evident. This represents the physiological lobule in man, which anatomically is not so distinct as in some lower animals. This could not now be called cirrhosis but the foreshadow is obvious. Hematoxylin-eosin stain. $\times 60$.



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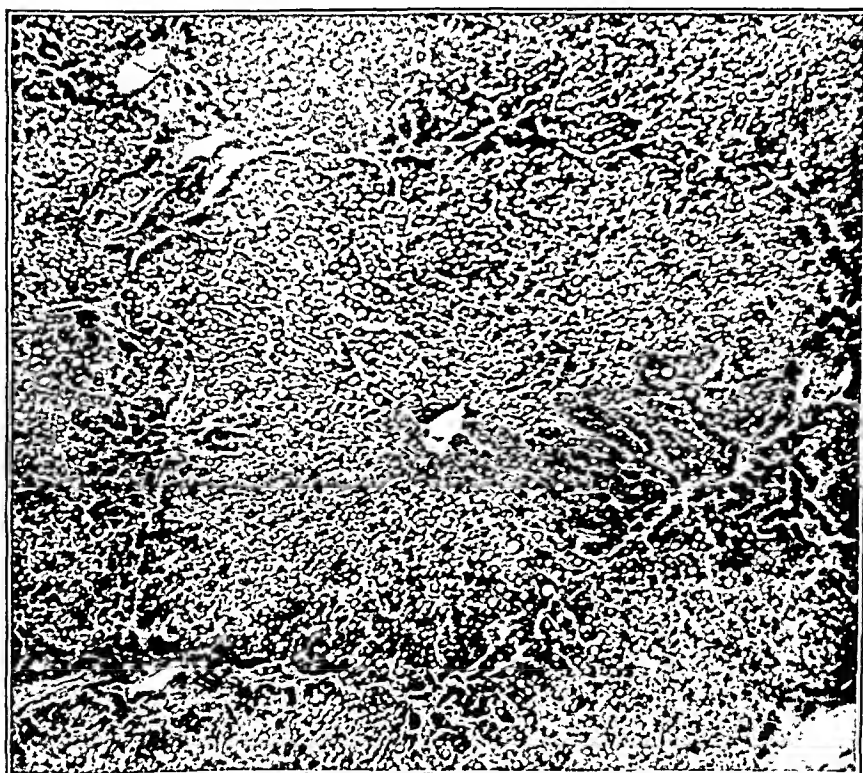
PLATE 71

FIG. 5. Microphotograph showing the beginning of cirrhosis in a fatty alcoholic liver. A wall of fibrous tissue now surrounds the lobule forming an anatomical as well as a physiological unit. Hematoxylin-eosin stain. $\times 60$.

FIG. 6. Microphotograph of liver showing early but definite cirrhosis of the liver of the rapidly progressing alcoholic type. This section contains all the cellular and tissue changes which characterize the histogenesis of alcoholic cirrhosis. Modification of Masson's trichrome stain. $\times 60$. These are shown in Figs. 7, 8 and 9. Figs. 6, 7, 8 and 9 are from Case 7.



3



4

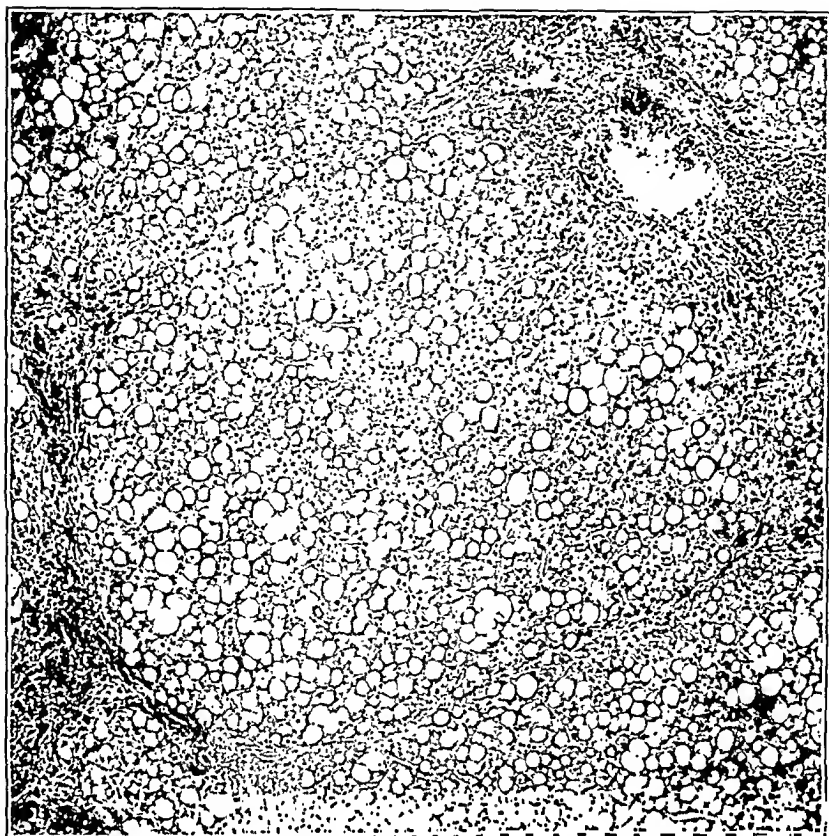
PLATE 72

FIG. 7. Microphotograph of liver showing atrophy and degeneration of cells at the periphery of a lobule in "acute" cirrhosis. Hematoxylin-eosin stain. $\times 500$.

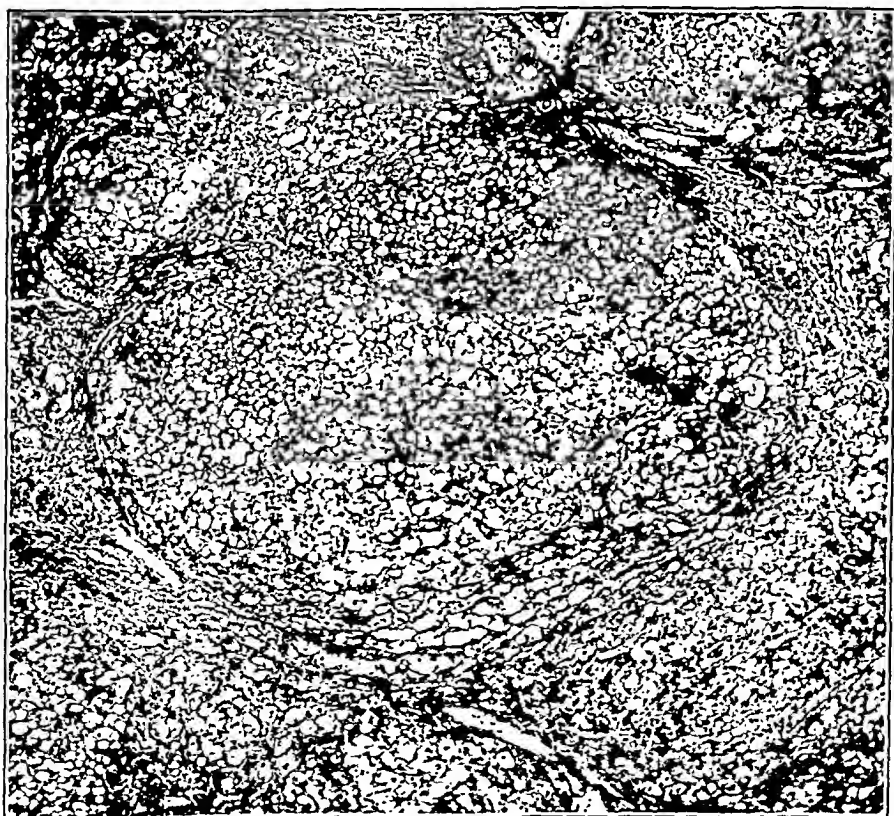
FIG. 8. Microphotograph showing the hyaline clumps described by Mallory, which are present in progressive cirrhosis but absent in the quiescent phases. Fatty infiltration, fatty degeneration and cell atrophy can be seen also. Proliferating stroma not easily recognized by this technique. Picture taken with green color filter to bring out hyaline masses in cells undergoing degeneration. Hematoxylin-eosin stain. $\times 500$.

FIG. 9. Microphotograph of liver showing the development of reticular and collagen fibrils around a lobule and between cells, following the natural line of cleavage along a bloodless capillary. Masson's trichrome-silver method. $\times 600$.

FIG. 10. Microphotograph of liver showing well advanced cirrhosis with some residual fat and clumps of atrophic cells persisting in the fibrous tissue. From a seaman, 50 years of age, who drank a great deal of whiskey at intervals, and some all the time. Three years before death he had been in the same hospital with vomiting, jaundice and an enlarged liver. On his last admission there was no jaundice, the liver was smaller, and he presented the typical findings clinically and at autopsy of cirrhosis of the liver. Hematoxylin-eosin stain. $\times 50$.



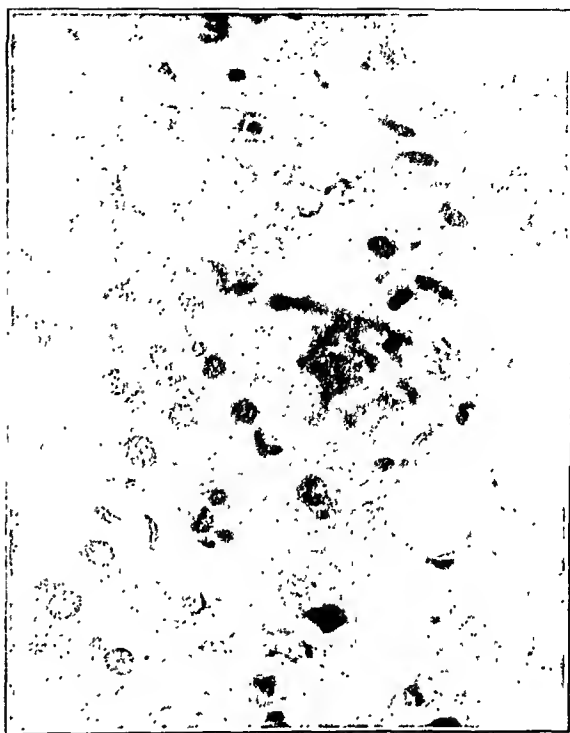
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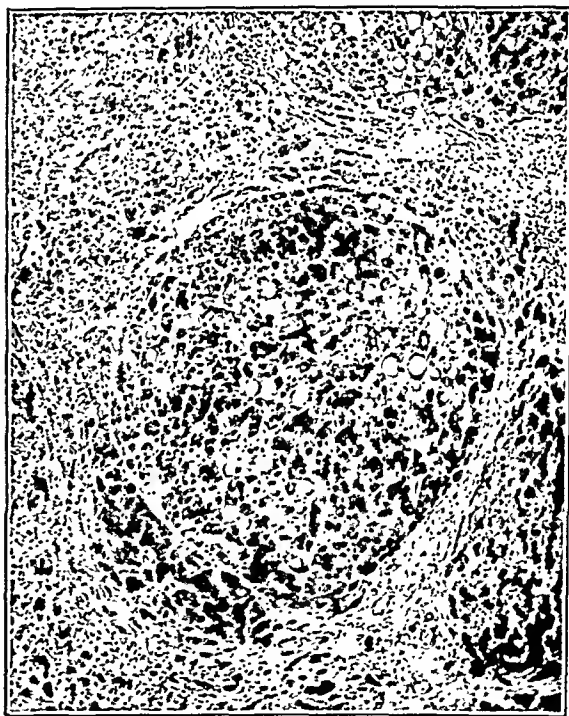
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Connor

Fatty Infiltration of Liver

important tumor of lymph glands and states that it occurs in connection with lymphatic leukemia. A characteristic feature of lymphomas is their simultaneous appearance in numerous lymphatic glands as well as in the lymph follicles of various organs. At the same time diffuse leukemic infiltration may occur in organs such as the liver and kidneys which do not belong to the lymphatic system.

Pseudoleukemia, in which anatomical changes similar to those of true leukemia occur in the spleen, bone marrow, lymphatic glands and other parts of the body without any change in the numerical ratio of the white or red corpuscles, is much more common than true leukemia in food-producing animals, according to most investigators. The term lymphogranulomatosis or false pseudoleukemia has been applied to Hodgkin's disease or Sternberg's pseudoleukemia as in this affection the nodules in the lymphatic glands, spleen, liver, kidneys and lungs consist of typical granulation tissue formed of plasma cells, lymphocytes, leukocytes and Sternberg's giant cells. On the evidence of this histological picture, the occurrence of Hodgkin's disease in food animals has not been proved, but MacMahon,² and Stalker, Schlotthauer and Feldman³ have encountered this disease in dogs, while Medlar and Sasano⁴ reported a neoplasm in a rabbit which corresponded closely to Hodgkin's disease in man.

Lymphosarcoma is thought to be common in animals and bears a gross resemblance to leukemia. It is described as beginning with tumor formations in the lymphatic glands, leads to infiltration of the surrounding tissue, and may give rise to numerous lymphatic nodules by way of the lymph stream, and also form metastatic deposits in the spleen, liver and bone marrow. Therefore, it is said to occupy an intermediate position between pseudoleukemia and malignant tumors. Lymphosarcoma of dogs, which is transmissible from animal to animal, is thought to be a true tumor formation, manifested by multiple tumors formed of small round cells resembling lymphocytes. It is considered by some investigators to be transmitted by a filterable virus.

Many comparative pathologists believe that the primary types of progressive lymphoid hyperplasia are true neoplasms and consider them so closely related genetically as to justify their classification in one group known as lymphoblastoma, as suggested by

PRIMARY RETICULUM CELL SARCOMA OF THE LYMPH NODES OF A COW WITH WIDESPREAD METASTASES *

JOHN S. BENGSTON, PH.G., D.V.M.

*(From the Branch Pathological Laboratory, Chicago, Illinois, Pathological Division,
Bureau of Animal Industry, United States Department of Agriculture)*

INTRODUCTION

Conditions resembling lymphoid hyperplasia are frequently observed in food-producing animals and are usually thought to be a leukemia or leukemic-like disease arising from the lymphoid elements or the cells of the lymphocytic series. European investigators have from time to time made studies of leukemia and leukemic-like diseases in such animals, but it appears that comparatively little investigation has been done along this line in the United States. A review of the literature presents a bewildering discussion dealing with lymphatic leukemia, pseudoleukemia, lymphadenoma, leukemia, lymphoblastoma, lymphocytoma, lymphosarcoma, alveolar sarcoma and round-cell sarcoma. It appears that the various authors in describing cases belonging to this group of diseases have selected from these terms those which they thought most appropriate for their particular cases without making any detailed or intensive study of the underlying histopathological changes. Furthermore, the opportunity for making such detailed studies on the living animal has been rather infrequent because this group of diseases is rarely detected in the live animal and is generally first seen after the animal has been slaughtered for food purposes. In emaciated animals it may sometimes be possible to recognize these so-called lymphoid hyperplasias due to the prominence of greatly enlarged superficial lymph nodes, but the vast majority of these conditions are not detected or even suspected in the various food animals until after they are slaughtered and a postmortem examination made.

In Germany Ostertag¹ has written considerably concerning these lymphoid hyperplasias or so-called leukemias in food-producing animals. He considers lymphoma, on account of its frequency and importance in meat inspection, to be the most

* Received for publication December 29, 1937.

various tissues and organs affected with lymphoid hyperplasia sufficient evidence to distinguish between leukemic and aleukemic conditions. I doubt the wisdom of such a practice as I do not think the condition of the blood in the vessels of cut and stained sections of tissues affords definite evidence of the condition of the circulating blood in the live animal.

I have studied many cases of these so-called leukemias, or lymphoid hyperplasias, in the different species of food-producing animals with a view of obtaining more definite knowledge concerning them. Various obstacles have interfered with a complete detailed study of these cases. In the first place opportunity was not afforded for making a clinical examination of the live animal with blood studies, (2) some other person usually performed the postmortem examination and selected the tissues for study, and (3) the tissues submitted for examination were frequently not fresh enough for a detailed cytological study.

A supervising inspector recently called our attention to a cow that had a number of the superficial lymph glands greatly enlarged. This cow was one of a lot of cattle received at the Chicago stockyards for slaughter. After clinical and histopathological studies of the case the conclusion was reached that the animal was affected with a primary reticulum cell sarcoma of the lymph glands with widespread metastases. Because such cases have not previously been reported in veterinary literature (so far as I am aware) the case is considered of sufficient interest and importance to warrant reporting in detail.

REPORT OF CASE

Clinical Findings and Blood Examination: The subject was a large Holstein cow approximately 8 years of age and in good flesh. The animal walked with an even steady gait but the prescapular and precrural lymph nodes stood out very prominently. The left prescapular lymph node appeared to be about 18 by 26 cm., while the right prescapular lymph node and both precrural lymph nodes appeared to be about 7 cm. in diameter. No other enlarged lymph nodes could be detected on palpation. A number of blood smears were prepared from blood drawn from an ear vein and stained with Wright's and Giemsa's blood stains. On microscopic examination these blood smears were normal. No abnormal erythrocytes were

Mallory.⁵ In the Federal Meat Inspection Service these malignant and metastatic lymphoid hyperplasias are usually grouped under the collective term "pseudoleukemia." All species and ages of food-producing animals are found to be affected. Condemnations for pseudoleukemia, as published in the report of the Chief of the Bureau of Animal Industry of the United States Department of Agriculture, for the fiscal year ending June 30, 1936, are given in Table I.

TABLE I

Comparison of the Number of Food-Producing Animals Condemned for Pseudoleukemia with the Total Number of Animals Condemned on Postmortem Examination by the Federal Meat Inspection Service of the Bureau of Animal Industry During the Fiscal Year Ended June 30, 1936

Species	Total number examined	Total number condemned	Per cent condemned	Condemned for pseudoleukemia	Per cent condemned for pseudoleukemia
Cattle	10,298,213	69,595	0.67	1385	0.0134
Calves *	5,783,154	19,340	0.33	50	0.001
Sheep	17,316,665	36,028	0.20	20	0.000115
Goats	51,464	185	0.36	1	0.07
Swine	28,506,019	86,820	0.30	180	0.00063
Horses	14,899	136	0.90	0	0.0

* Aged 1 year or less.

Undoubtedly cases of true leukemia do occur among food-producing animals and a number of cases thought to be true leukemia have been reported in the literature, but many of them are open to question because of insufficient cytological study. Creech and Bunyea⁶ studied a case in a cow in which the definite changes in the cell ratio proved to be a true leukemia, the white cell count reaching a level of 203,250. The case reported by Gray⁷ as lymphatic leukemia in a 5 year old Jersey cow lacks convincing proof that it actually was a true leukemia. Feldman⁸ prefers to place the conditions known in veterinary literature as leukemia, pseudoleukemia, malignant lymphadenoma, lymphosarcoma, and many others, under the designation of lymphocytoma. Experienced veterinary pathologists admit that it is difficult to distinguish between leukemia, pseudoleukemia, and allied conditions, either macroscopically or microscopically. Some investigators consider the presence of an excessive number of white blood cells in the blood remaining in the vessels of cut and stained sections of

various tissues and organs affected with lymphoid hyperplasia sufficient evidence to distinguish between leukemic and aleukemic conditions. I doubt the wisdom of such a practice as I do not think the condition of the blood in the vessels of cut and stained sections of tissues affords definite evidence of the condition of the circulating blood in the live animal.

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observed and there was not an excessive number of leukocytes present.

The cow was bled again 2 days later for the purpose of making further blood counts. A test for hemoglobin was also made at this time, using the Tallqvist hemoglobin scale, and a reading of about 65 was obtained. Blood counts made from the oxalated blood from the jugular vein revealed about 6,000,000 erythrocytes and 6900 leukocytes per cmm. with 31 per cent polymorphonuclears, 51 per cent lymphocytes, 9 per cent monocytes and 9 per cent eosinophiles. The cow was slaughtered and autopsied on the following day.

POSTMORTEM FINDINGS

The carcass was that of a fairly well nourished mature cow with a normal fat content and distribution for this particular type of animal.

Head: The mandibular, parotid, atlantal and retropharyngeal lymph glands were moderately enlarged and grayish white in color. They were somewhat softer in consistence than normal.

Lungs: The lungs and associated lymph glands were essentially normal in appearance and consistence. The parietal and visceral pleura were smooth and glistening. In the anterior portion of the thoracic cavity on the left side was a mass of tissue measuring 10 by 6 cm. This mass of tissue was grayish white and moderately soft, but contained no recognizable thymic tissue.

Heart: The emptied heart weighed 4300 gm., which is about normal for an animal of this size. The myocardium was firm but contained a large amount of a grayish white tissue, which extended through the entire wall of the myocardium. Both auricles were almost completely replaced with a similar grayish white, moderately firm tissue.

Liver: The liver was normal in size, consistence and color.

Spleen: The spleen was normal in size but was firmer than usual. The capsule was thickened and fibrous tags adhered to it. The splenic pulp was normal in appearance.

Kidneys: Both kidneys were enlarged, each measuring 22 by 14 by 8 cm. Extending above the surface of both kidneys were a number of large, light colored nodules which upon being incised were found to contain copious amounts of a yellow semifluid pus.

Only a thin shell of kidney tissue remained at the periphery. This condition was diagnosed as bilateral pyonephrosis.

Intestines: The small intestines contained throughout their entirety innumerable nodules or deposits in their walls, which were more noticeable as a rule on the serous than on the mucous surface, although many nodules were distinctly visible on the mucous surface and protruded for varying distances into the lumen of the intestines. These nodules measured up to 6 cm. in diameter and were grayish white, moderately firm and had a more or less irregular surface contour. The whole chain of mesenteric lymph glands was markedly increased in size, grayish white and moderately firm. On incision they appeared moist. Practically all the visceral lymph glands in the abdominal cavity were enlarged and resembled the mesenteric lymph glands in color and consistence.

Uterus: The uterus contained what was approximately a 5 months fetus. The whole uterine wall was greatly thickened, measuring as much as 3.5 cm. in some places. This thickened uterus was very pale in color, almost a grayish white and was moderately firm. Both ovaries appeared normal. The various internal organs and lymphatic glands of the fetus showed no visible abnormalities.

Skeletal Muscle: In the muscles of the flank on the right side a grayish white area measuring 16.5 by 7.5 cm. was noted. This grayish white area replaced the muscles in the area involved and was moderately firm.

Lymph Glands: The left prescapular lymph gland was enormous in size and adherent to the surrounding tissues. It measured 43 by 32 by 20 cm. Sisson⁹ gives the normal dimensions of this gland as 10 to 12 cm. long and 3 cm. wide. The gland was grayish white with a slight mottling of red and contained a few small necrotic areas. It was moderately firm and the cut surface appeared somewhat moist. The right prescapular lymph gland was also enlarged, measuring 24 by 12 by 10 cm., and resembled the left prescapular lymph gland in appearance and consistence. The left precrural lymph gland measured 28 by 14 by 13 cm., and the right precrural lymph gland measured 18 by 8 by 6 cm. Sisson⁹ gives the normal dimensions for these glands as 8 to 10 cm. long and 2.5 cm. wide. The color and consistence of these glands were similar to those of the prescapular lymph glands.

observed and there was not an excessive number of leukocytes present.

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Kidneys: Both kidneys were enlarged, each measuring 22 by 14 by 8 cm. Extending above the surface of both kidneys were a number of large, light colored nodules which upon being incised were found to contain copious amounts of a yellow semifluid pus.

cell of this type was seen among the tumor cells in all sections. In some blood vessels and lymphatic channels of the lymph nodes they were seen in clumps and groups. This same erythropoiesis was also seen in places where it was not associated with the neoplastic changes, for example in the spleen capsule. It did not appear to be an essential part of the tumor process.

Lymph Glands: The normal architecture was completely destroyed by an overgrowth of tumor cells. Only an occasional sinus and blood vessel was recognizable. The capsule was slightly thickened by dense fibrous connective tissue and was heavily invaded by the anaplastic tumor cells.

Heart: The heart was diffusely invaded by the tumor cells from the epicardium to the endocardium. They did not push the muscle fibers aside but actually destroyed them by invasion. The muscle thus invaded showed various stages of degeneration.

Skeletal Muscle: The same characteristic feature noted in the myocardium was also seen in the skeletal musculature in that the tumor cells actually invaded and destroyed the muscle fibers rather than by spreading the muscle fibers apart. Sheets of tumor cells were present in the invaded muscle. The muscle fibers showed atrophic and degenerative changes.

Intestine: The intestinal nodules were composed of tumor cells of the type already described which had invaded and destroyed the entire muscularis mucosa and mucosa. Only remnants of the glandular and muscle structures remained. Although these nodules were fairly well circumscribed, they could be seen projecting into the surrounding intestinal wall. Adjacent to the nodules the mucosa was heavily infiltrated by lymphocytes, plasma cells and leukocytes, especially eosinophils.

Uterus: The entire wall was to a large extent replaced by tumor tissue as above described and consequently was greatly thickened.

Liver: The normal architecture was well preserved. The periportal areas showed a moderate increase in cellularity, mainly of histiocytic proliferation. The Kupffer cells were numerous but showed no definite phagocytosis. In the sinusoids there was noted an occasional bone marrow giant cell or lymphocyte. Small nests of rather large cells with vesicular nuclei and the chromatin arranged around the periphery of the nuclear capsule were found scattered irregularly throughout the liver. Some of the liver cells

Bone Marrow: The bone marrow in the medullary cavities in the diaphyses of the tibia, femur and humerus was found to be of a normal yellow fatty consistence. The bone marrow of the ribs was deep red in color.

Tentative Anatomical Diagnosis: The lesions noted in this case were considered typically characteristic of the condition in cattle commonly designated pseudoleukemia.

HISTOPATHOLOGY

The neoplasm was essentially the same in all sections, consisting of an intermingling of two types of cells. One of these which predominated in all parts of the growths had features of malignancy and was considered the essential cell. It was identified as a malignant reticulum cell. These cells grew in sheets or formed irregular alveoli of cells and infiltrated other tissues freely. They varied in size and in shape and were from 15 to 30 μ , or even more, in diameter. The cytoplasm was moderately abundant, clear, without specific granulations, and slightly oxyphilic in staining. When not altered by pressure relations the cells tended to be oval, although the cytoplasm often streamed off into points. The nuclei occupied the major portion of the cells and tended to be oval, although many were kidney shaped, lobulated or knobbed. The chromatin in the nuclei varied greatly in amount and distribution, but on the whole was very abundant. In some of the cells the chromatin of the nuclei was in the form of coarse granules while in others it was finely distributed. The nuclei tended to be vesicular. Nucleoli were visible when the chromatin was not excessive. They were large, slightly acidophilic, and varied in number from one to three. Mitotic figures were numerous in the various sections, over twenty being visible in some high power fields. They were often abnormal and bizarre in character, showing unequal division of chromatin. Foot's modified silver method for reticulum fibers showed a uniform fine network so that nearly every tumor cell was conceivably in direct contact with a fibril. These tumor cells were phagocytic for other cells and cell débris in places where there was active cell disintegration.

The second type of cells seen in small numbers in all sections was of the erythropoietic series. These cells were polychromatophilic erythroblasts, erythroblasts and normoblasts. An occasional

cific diseases based on their clinical course and microscopic picture. Concerning the histogenesis of these tumors Ewing states that the reticulum cells of the lymph nodes are derived from connective tissue cells and form a meshwork in which lymphocytes gather from without. The reticulum of the follicles and pulp does not produce lymphocytes but these two cells constitute separate series. From the reticulum cells of the follicles can be traced the development of large round cell tumors of the type of large round and giant cell lymphosarcoma, which must be separated from lymphocytoma. The tumors composed of loose round cells derived from the reticulum of the follicles differ markedly from the others, and it seems unwise to urge any change in their usual designation as reticulum cell sarcoma.

Ewing does not mention the argyrophilic reticulum, which is a constant feature of tumors of reticuloendothelial origin. In the microscopic diagnosis of reticulum cell sarcoma of the lymph nodes silver impregnation and fat stains are obligatory in order that the characteristic argentophil fibrils and lipoids may be brought out. Based on extensive study of neoplastic conditions Callender,¹¹ registrar of the American Registry of Pathology, regards the blood monocyte and the reticuloendothelial cell as being one and the same cell, and states that the reticulum cell sarcoma really includes Hodgkin's sarcoma and is a better term for the condition.

"Retothelial sarcoma" is a term suggested to Roulet by Rössle, who used it in articles published in 1930 and 1932 to replace the term "reticulum cell lymphosarcoma." The derivation is from the German "Retothelien" (reticulum). The term is more accurately used, however, as a contraction of the bulky term reticuloendothelial because the reticular element should not be separated from the endothelial element in this system. Reticulum cell lymphosarcoma, or rethothal sarcoma, was recognized as a type of lymphosarcoma by Ghon and Roman in 1916. Because the relation of the lymphoblast to the reticulum cell was not then clear, it had been considered as related to lymphoblastic lymphosarcoma, but Roulet clearly demarcated it from the latter.

Sarcomas as a rule do not invade the lymphatics but metastasize by way of the blood stream; but lymphatic and reticulum cell lymphosarcoma, which originate in lymph nodes, may travel by

showed hydropic degeneration. Some of the larger blood vessels contained lymphocytes and occasional bone marrow giant cells. Many of the sublobular veins contained large numbers of mononuclear cells and some lymphocytic and monocytic cells. There was no tumor infiltration in the liver.

Spleen: The follicles were well preserved and distinct but showed no definite germinal centers. The pulp showed a marked increase of fibrous tissue. The spleen also showed much golden brown pigment in macrophages, and erythropoiesis in the sinusoids and red pulp. The capsule was thickened by dense fibrous connective tissue. A fibrous tag attached to the capsule was traversed by large sinuses, some of which were filled with red cells, lymphocytes and blood pigment. There was no tumor infiltration in the spleen.

Bone Marrow: The red bone marrow of the rib showed active granulopoiesis and hematopoiesis. There were various stages from erythrogonia to normoblasts and erythrocytes as well as myeloblasts and metamyeloblasts and granulocytes. Bone marrow giant cells were found throughout, some having innumerable lobed nuclei. This was a very active but normal bone marrow.

Final Anatomical Diagnosis: Primary reticulum cell sarcoma of the lymph nodes with metastases in the heart, intestines, uterus and skeletal muscles.

DISCUSSION

No evidence of myelopoiesis was found in any of the tumor sections studied and there seems to have been no tendency for the cells to mature in the direction of myelocytes. Extramedullary erythropoiesis is very common in the spleen, liver, lymph nodes, kidney, and so on, in instances of carcinoma as well as in other chronic diseases, but it is not usually seen in the tumor itself, as in this case.

No proliferation or participation of the vascular endothelium in the tumor process was noted, and all blood vessels apparently resisted invasion by the tumor cells.

Ewing¹⁰ distinguishes between endothelioma of lymph nodes arising from the endothelial lining of lymph and cavernous sinuses, and a loosely arranged, large celled lymphosarcoma arising from the reticulum cells of the lymph nodes. He regards them as spe-

ances of the lymphoid elements and diagnosed as leukemia, pseudoleukemia, lymphosarcoma, lymphocytoma, and so on.

Note: I wish to express my appreciation to Dr. S. R. Rosenthal and to Dr. P. E. Steiner for the valuable advice and information which they so cheerfully furnished while making these studies.

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DESCRIPTION OF PLATES

PLATE 73

FIG. 1. Cross section of precrucial lymph node of a cow affected with reticulum cell sarcoma compared with a normal precrucial lymph node.

FIG. 2. Nodular formations of reticulum cell sarcoma in the wall of the small intestine of a cow.

way of both lymph and blood vessels, so that these tumors may yield the most abundant and widely disseminated metastases seen with any type of neoplasm. The leukemic equivalent of reticulum cell sarcoma, namely a monocytic leukemia, should eventually be discovered in food-producing animals.

SUMMARY AND CONCLUSIONS

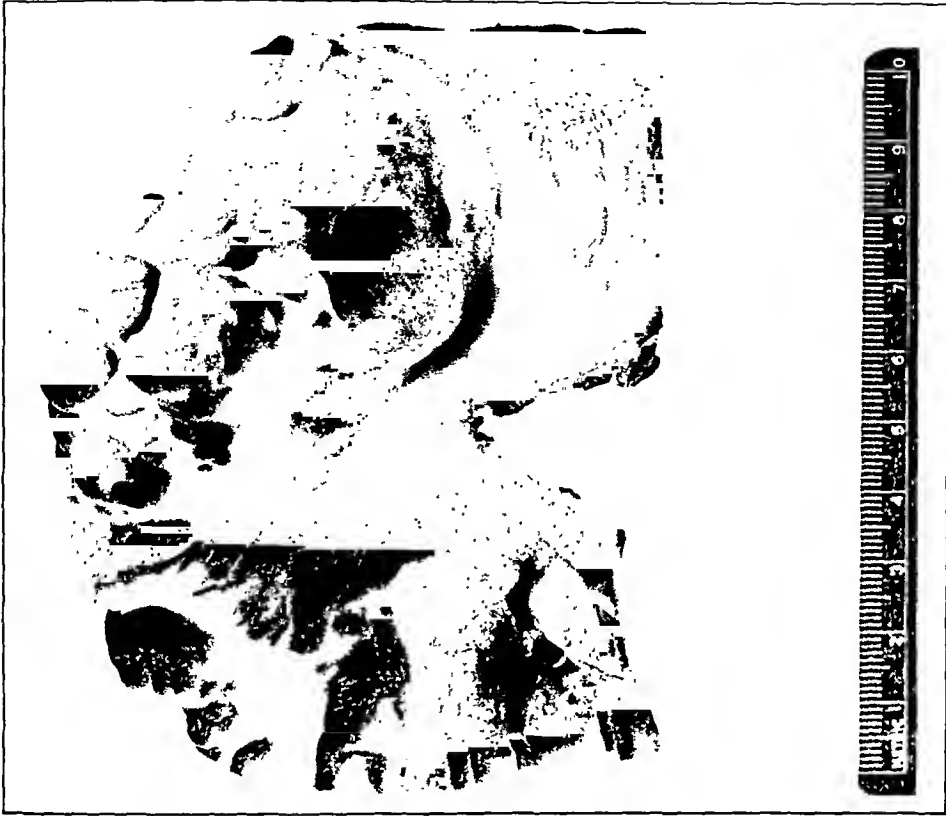
The case of an 8 year old Holstein cow affected with greatly enlarged superficial lymph nodes is presented. Clinical examination of the blood revealed a hemoglobin index of 65 (Tallqvist), and normal white, differential and red cell counts. Postmortem examination revealed widespread enlargement of the lymph nodes with tumor formations, considered to be metastases, in the heart, intestines, uterus and skeletal muscles. The lungs, liver, spleen and bone marrow were not invaded by tumor growth. Microscopically the enlarged lymph nodes and tumor growths in the heart, intestines, uterus and skeletal muscles were identical, being composed principally of large, irregularly shaped cells measuring up to 30μ or more and containing large, oval, kidney shaped, lobulated or knobbed nuclei which tended to be vesicular. Mitotic figures were exceptionally numerous, twenty or more being visible in some high power fields. Silver impregnation revealed a uniform fine network of argyrophil fibers virtually in direct contact with every tumor cell. Many of the tumor cells were phagocytic for other cells and cell debris. Scattered throughout the tumor cells were small areas of erythropoiesis which did not appear to be an essential part of the tumor process. The tumor cells proper in this case are believed to be malignant reticulum cells having their origin in the reticulum of the lymph nodes. These reticulum cells are not lymphocytes but are of connective tissue origin, and tumors originating from such reticulum cells constitute a specific group that must be separated from lymphocytoma, pseudoleukemia and lymphatic lymphosarcoma.

Although reticulum cell sarcoma has not been reported previously in food-producing animals, I do not believe this is a rare neoplasm. When fresh tissues are obtained and sections are appropriately stained and the silver impregnation method used, it is believed that many cases of reticulum cell sarcoma will be diagnosed in animals which were formerly considered to be disturb-

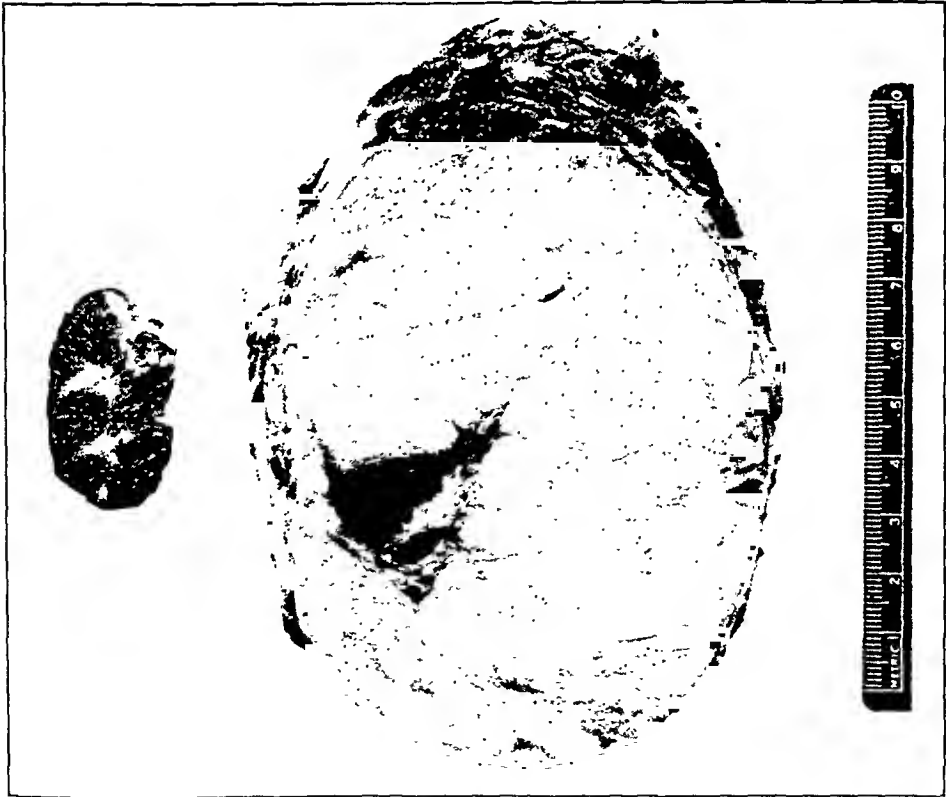
PLATE 75

FIG. 5. Section of lymph node affected with reticulum cell sarcoma. Note the irregularly shaped tumor cells and nuclei. Several mitotic figures are shown. $\times 1800$.

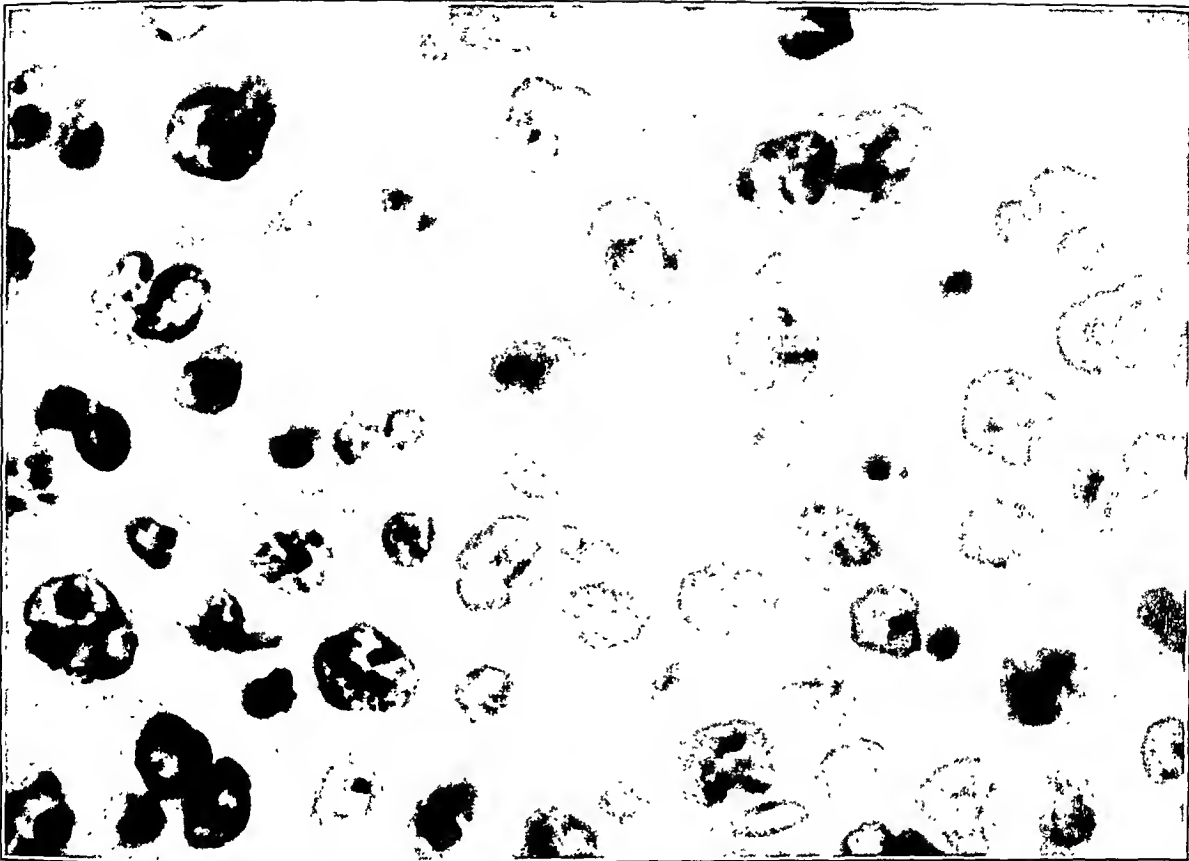
FIG. 6. Same lymph node as shown in Fig. 5 impregnated with silver to show the network of delicate argyrophilic fibrils in direct contact with the tumor cells. $\times 775$.



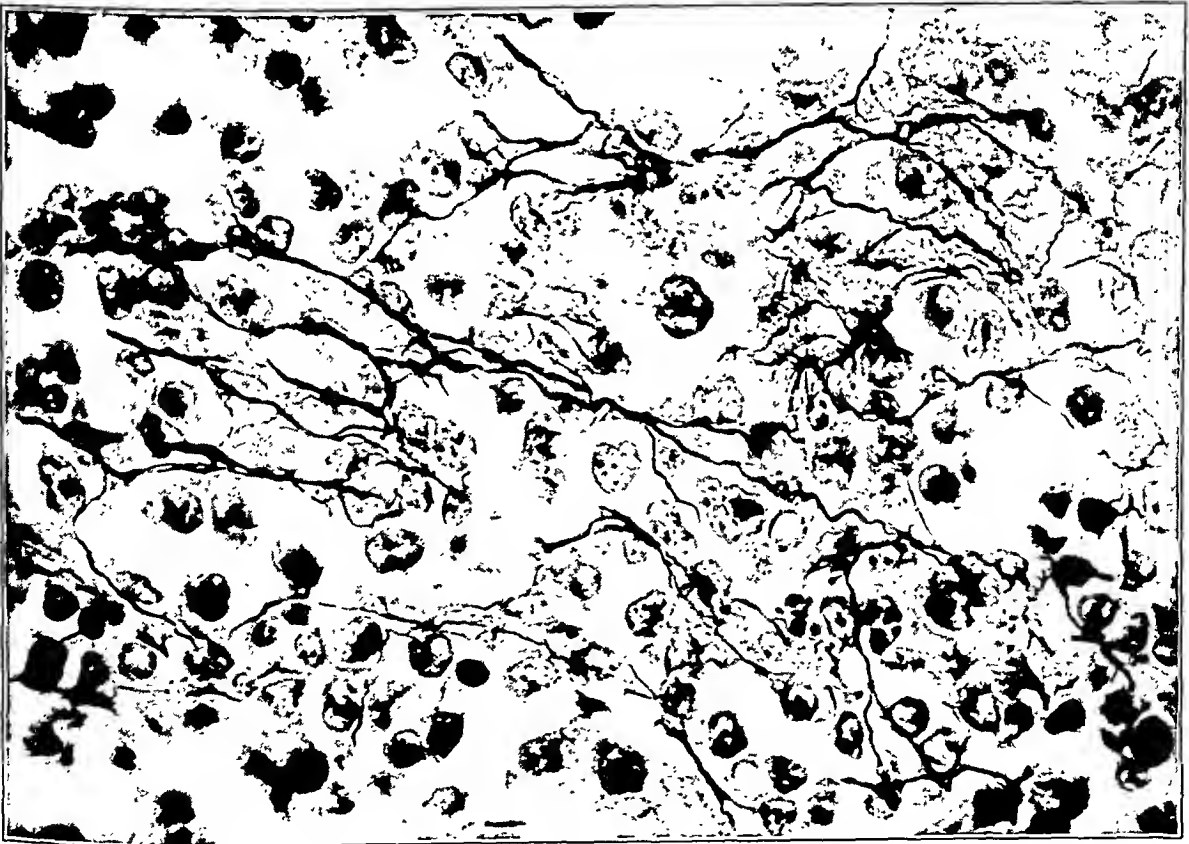
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observations on the degree of leukocytosis that results from the infection.

METHOD

A standard suspension of *H. pertussis* is prepared by scraping a 72 hour growth from the surface of Bradford's medium¹² into 0.85 per cent NaCl solution. It is standardized to contain approximately 10 billion organisms per ml.

White mice, weighing from 15 to 20 gm., are anesthetized with ether in a glass jar. A small longitudinal incision is made over the ventral surface of the neck and the muscles retracted. By means of a bent probe, the trachea is brought into view and 0.05 ml. of the standard suspension of *H. pertussis* is introduced through a small needle from a tuberculin syringe. The cut edges of the skin are approximated and held in place by the application of a small amount of collodion. The entire procedure requires only a few minutes and less than 5 per cent of the mice are killed by it.

RESULTS

With the dosage employed, from 60 to 75 per cent of the mice die after inoculation within from 1 to 10 days. The organism can be consistently recovered from the lungs, frequently in pure culture, for as long as 10 to 20 days after inoculation. During the first 3 days of infection, invasion of the blood stream occurs, as may be proved by making cultures of the peripheral blood from the tail, from the heart's blood, and from the spleen.

A definite respiratory wheeze, frequently persisting for several hours, results from the inoculation. During the next 24 hours the mouse becomes less active, refuses food, loses weight, and presents a roughened coat. Animals that die within 48 to 72 hours closely resemble those suffering from virulent pneumococcal infection.

When the mice are autopsied there is often a small amount of free bloody fluid in the serous cavities and small petechial hemorrhages in the peritoneum and pleura. The liver, spleen and kidneys are enlarged. The lungs are distended and patchy or diffuse hemorrhagic areas of consolidation are noted, particularly over the hilum and apical regions (Fig. 1). These areas are not depressed and are firm and rubbery. On sectioning the cut surface

EXPERIMENTAL INFECTION IN THE MOUSE PRODUCED BY INTRATRACHEAL INOCULATION WITH *HEMOPHILUS* *PERTUSSIS* *

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Numerous attempts to produce experimental infection in animals with *Hemophilus pertussis* have been made. In certain instances, particularly in the puppy,^{1, 2} in the monkey,^{3, 4} and in the chimpanzee,^{5, 6, 7} the results have been encouraging. There can be little doubt that the disease has been experimentally established in the ape by Shibley⁶ and by Rich and his co-workers.⁷ MacDonald and MacDonald⁸ have reported that typical pertussis resulted when the upper respiratory tracts of two children were inoculated with suspensions of *H. pertussis*. Gallavan and Goodpasture,⁹ by inoculating the chorio-allantoic membrane or the amniotic fluid sac with *H. pertussis*, produced an experimental lung lesion in the chick embryo which they considered similar to that which occurs in the human disease.

During the past year we have been interested in the type of disease that results when the white mouse is inoculated intratracheally with recently isolated strains of *H. pertussis*. While the work was in progress Burnet and Timmins¹⁰ reported results obtained by the intranasal inoculation of mice. It is interesting that our findings are similar to theirs, apparently the only difference being in the method of inoculation and in the dosage. Although our method is a bit more laborious, we apparently obtain fewer secondary invading organisms by lung cultures. Culotta, Marting and Liebow¹¹ by intranasal inoculation compared the type of lesion produced in the young with that produced in the more mature mouse and described the pulmonary lesion as being "characterized by an accumulation of cells along the course of the broncho-vascular rays." We have observed this lesion in our animals and have also added

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TABLE I

Comparison of Total Leukocyte Counts in Mice Intratracheally Infected with Hemophilus pertussis and with an Atypical Pertussis-like Organism Isolated from Cases of Suspected Pertussis

Mouse	Organism	Total leukocyte counts					Results	Lung cultures
		Before inoculation	After inoculation			15 days		
			2 days	5 days	10 days			
A-1	<i>H. pertussis</i>	17,500	66,000	124,000		30,000	D14*	+
A-2	"	21,600	27,500	50,000			K28*	o
A-3	"	12,800	30,000	220,000	180,000		D10	+
A-4	"	16,000	25,000	39,000	84,500	67,500	K20	+
A-5	"	24,000	34,000	70,500			D10	+
A-6	"	18,500	27,000	44,500	18,500	11,000	K20	o
A-7	"	15,000	8,500				D2	+
A-8	"	21,000	36,000				D2	+
A-9	"	14,000	11,000	44,500	42,500		D15	+
B-1	Atypical pertussis-like organism	29,000	16,500	17,000			D15	o
B-2	"	18,000	38,000	62,000			D15	+
B-3	"	15,000	33,000	35,000		28,500	D22	o
B-4	"	16,500	23,500	6,500	26,500		K10	+
B-5	"	21,000	33,000	19,000	44,000		K15	+
B-6	"	22,500	45,000	45,000	19,500		K10	+
B-7	"	18,000	23,500	24,000	27,000		K15	o
B-8	"	14,500	19,500				K3	+
B-9	"	19,500	8,000				K5	+
C-1	Saline controls	13,500	24,500	16,500	24,000		K10	o
C-2	"	19,500	30,000	20,000	10,500		K10	o
C-3	"	20,000	28,000	24,000	22,500		K10	o

* D = died; K = killed; numerals represent the day after inoculation.

is moist but, in places, granular. The bronchi contain frothy exudate.

Microscopically, stained sections of the lungs reveal typical interstitial changes with thickening of the alveolar walls and infiltration of mononuclear and polymorphonuclear leukocytes about the blood vessels and bronchioles (Fig. 2). During the first 72 hours after inoculation considerable amounts of mucus and cellular exudate accumulate within the alveolar spaces. Edema is pronounced and atelectatic areas are almost always present. After 3 weeks there is still evidence of the interstitial changes but the degree of polymorphonuclear leukocytic invasion of the parenchyma is much less marked (Fig. 3). Considerable mucus covers the epithelium of the bronchi in which many clumps of minute bacilli may be demonstrated by bacterial stains (Fig. 4). Some proliferation of the epithelium of the bronchus is noted, producing a distorted irregular contour of the epithelial layer of cells, but desquamation and fibrinoid necrosis apparently do not often occur. In this respect the lung lesion does not resemble that which is said to be characteristic of experimental infection with influenza virus.¹³ Inclusion bodies have not been found.

HYPERLEUKOCYTOSIS

Counts made on blood samples obtained by clipping off a bit of the tail usually revealed a marked leukocytosis, frequently varying from 30,000 to 100,000 leukocytes per cubic millimeter (Table I). Normal counts by this method range between 10,000 and 20,000. The predominating type of cell is usually mononuclear, this type frequently representing 65 to 70 per cent of the total number of white cells. In the mouse the normal percentage of mononuclear cells in the blood ranges from 60 to 80 per cent.¹⁴ In one mouse (A-3, Table I) the total leukocyte count reached 220,000 on the 5th day after inoculation. This animal died on the 10th day and culture of the lungs yielded a pure growth of *H. pertussis*. Table I illustrates the degree of leukocytosis produced by infection with *H. pertussis* as compared with that resulting from infection with an atypical pertussis-like organism¹⁵ obtained from the upper respiratory tract of children suffering from pertussis. It is evident that the degree of leukocytosis is much greater in the mice infected with *H. pertussis*. Very little disturbance of the total

SUMMARY

By the intratracheal inoculation of mice with suspensions of recently isolated *Hemophilus pertussis*, a characteristic lung lesion has been produced. It consists of an interstitial pneumonia and the development of an excess of mucoid exudate within the smaller bronchi and alveolar spaces. The parenchymal lesion is more conspicuous than that of the bronchial epithelium.

Pure cultures of *H. pertussis* have been consistently obtained from infected lungs from 10 to 20 days after inoculation.

The total leukocyte count has been regularly increased and extreme degrees of hyperleukocytosis have been observed occasionally.

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white cell count resulted when 0.85 per cent NaCl solution was introduced into the trachea.

DISCUSSION

The type of lung infection in the mouse described in the present paper is apparently not specific for *H. pertussis*, because we have produced similar lesions with virulent strains of *H. influenzae* and with strains of atypical pertussis-like organisms that have some of the characteristics of *Brucella bronchiseptica*. In this respect, however, it may be recalled that Sprunt, Martin and Williams¹⁶ showed that interstitial lesions produced in the lungs of rabbits by intratracheal inoculations of *Bacillus typhosus* closely resembled those produced by *H. pertussis*. Through the courtesy of Dr. Goodpasture it has been possible to compare the mouse lesion with the experimental lesion in the chick embryo and with that characteristic of human pertussis. As shown by Gallavan and Goodpasture,⁹ the essential lesion in the chick embryo and in human pertussis is an invasion of polymorphonuclear leukocytes followed by necrosis of the basilar and midzonal area of the bronchial epithelium. Although we have observed some proliferation of the bronchial epithelium in the mouse, we have noted very little tendency of the polymorphonuclear leukocytes to invade this area. Necrosis of the epithelial cells has been observed but infrequently. On the other hand, we believe that the parenchymal lesion described above is more conspicuous and is the essential lesion produced by *H. pertussis* in the mouse lung.

The association of hyperleukocytosis with lung lesions in mice is interesting because the extremely high white cell count of the disease in the human is almost always associated with pneumonia. A comparison of the degree of leukocytosis produced by intratracheal with that produced by intraperitoneal inoculation of *H. pertussis* and a more detailed study of the degree of lymphocytosis is now being made.

That the mouse is susceptible to infection with *H. pertussis* is fortunate, because it affords an experimental infection that is readily available for study. Moreover, we have found this experimental disease useful in studying the virulence of different strains of *H. pertussis* and the protective qualities of various antigens and immune serums.

DESCRIPTION OF PLATES

PLATE 76

FIG. 1. A. Photograph of mouse lung showing typical 72 hour lesion produced by intratracheal inoculation of *H. pertussis*.

B. Photograph of normal mouse lung. $\times 2$.

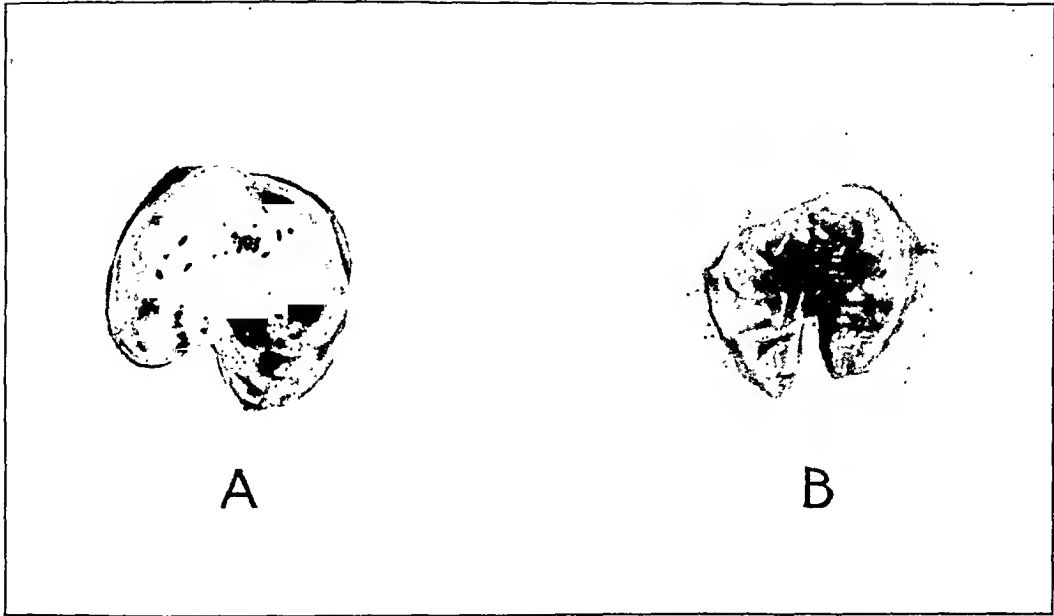
FIG. 2. Microphotograph of 72 hour lung lesion produced by intratracheal inoculation with *H. pertussis*, showing extensive alveolar and peribronchial cellular reaction and excessive exudate in the alveolar and bronchial lumens. $\times 210$.

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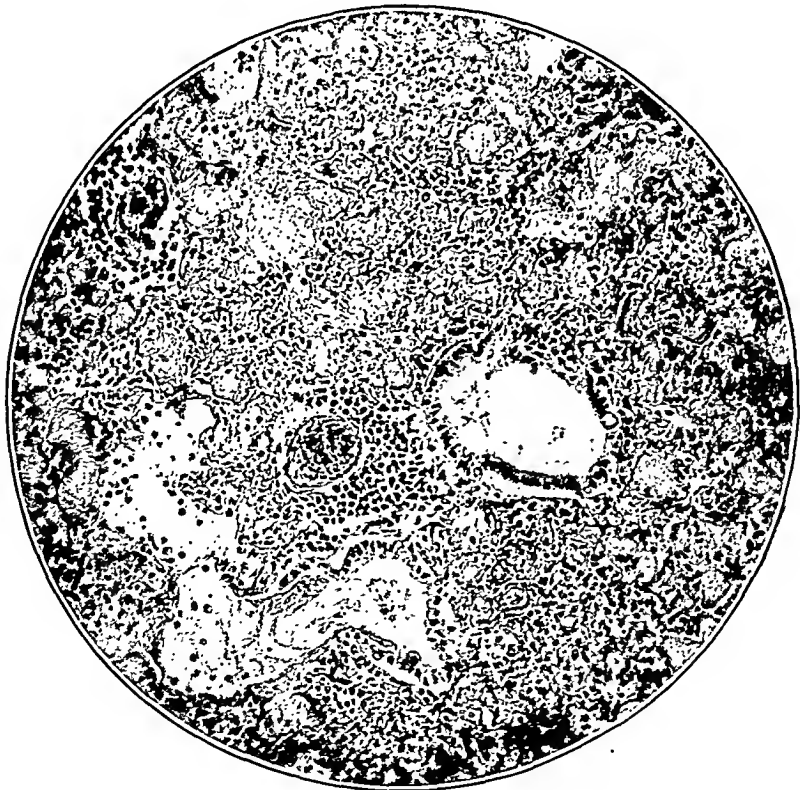
PLATE 77

FIG. 3. Microphotograph of 20 day lung lesion showing interstitial changes and cellular infiltration. $\times 210$.

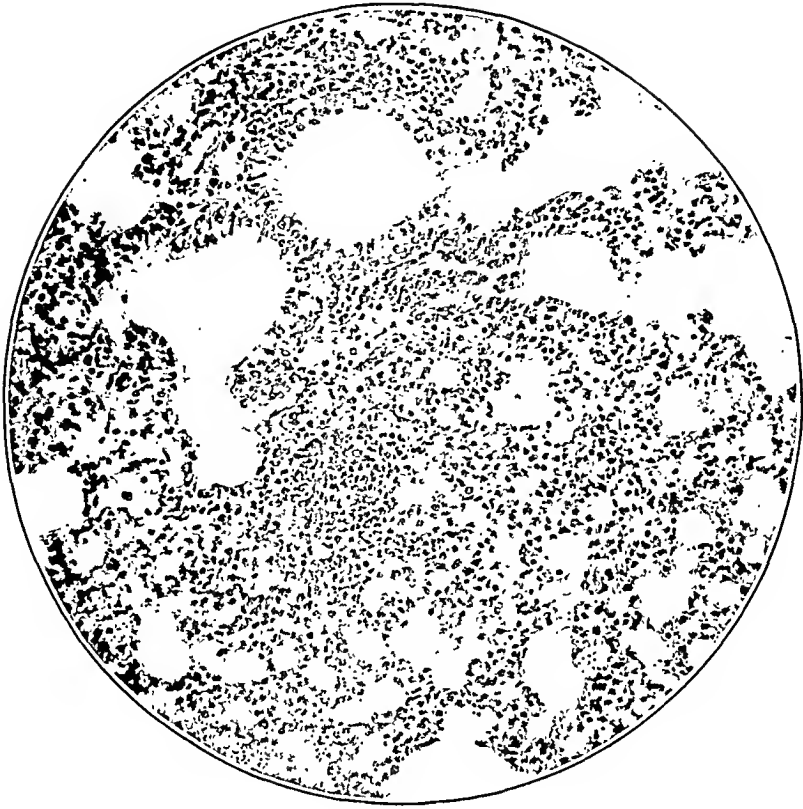
FIG. 4. Microphotograph of 20 day lung lesion. Bacterial stain showing clumps of *H. pertussis* in the bronchial epithelium. Pure culture of *H. pertussis* obtained from the lungs at autopsy. $\times 1500$.



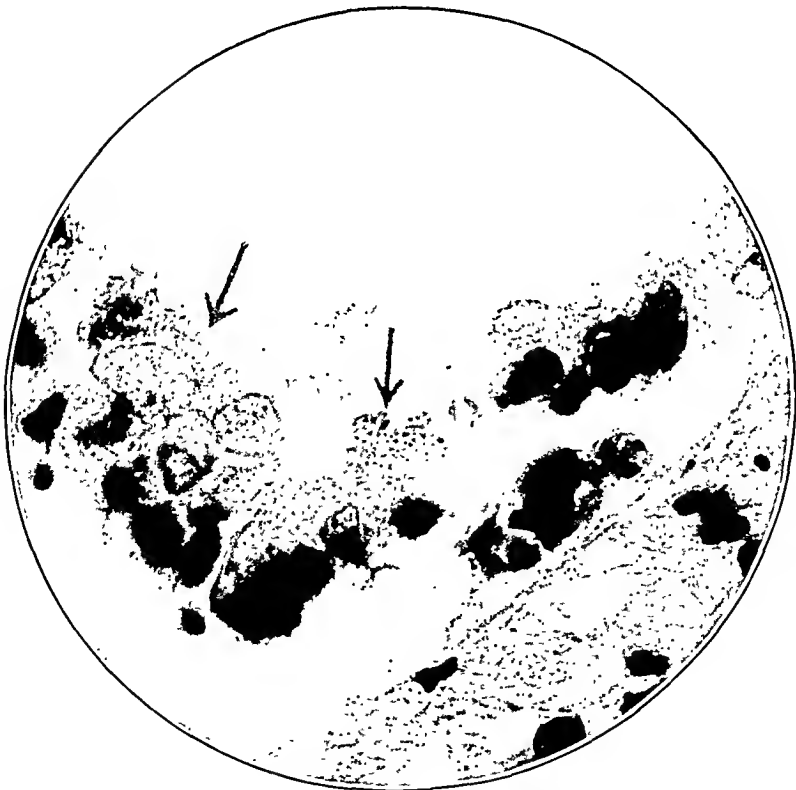
I



2



3



4

The data supporting the implantation theory for the origin of the secondary tumors of the tubal mucosa in these 7 cases are: the tubal carcinomas were superficial in character and presented the appearance either of having arisen from the epithelium of the mucosa or of having been added to it; their histological structure was similar to that of many of the implantation carcinomas on the lining of lymph vessels and on the peritoneal serosa; in all but one instance they were not only multiple but varied in size, thus suggesting different ages; evidence was present in each instance indicating that cancer cells from the uterine growth could easily have escaped into the lumens of the tubes; clumps of cancer cells were found in the lumens of the tubes; on the other hand carcinoma was found in the lymphatics of these 7 tubes in only one instance (Case 11 of that paper¹) and well may have arisen from the invasion of the fimbrial mucosa by carcinoma implanted on its surface.

Since the tubal carcinomas presented the histological picture either of arising from the mucosa or of having been added to it, the possibility of a multicentric origin for some or even all of the tubal tumors could not be excluded.

Newly formed tissue on the surface of the tubal mucosa with cancer cells enmeshed in it was found in only one instance (see Fig. 77 of previous paper¹). The failure to find more evidence of newly formed tissue on the surface of this mucosa, as well as the failure to detect all the stages in the implantation of cancer cells on the tubal mucosa in this series of cases, aroused my curiosity and urged me to study further the implantation of cancer cells in this situation. This is the real incentive for the study of the problem of the present paper.

If cancer cells actually become implanted on the surface of the tubal mucosa, the secondary tumors should not only resemble carcinomatous implants in other situations but all stages in their pathogenesis must be demonstrated. Otherwise this theory cannot be accepted.

If cancer cells escaping from a uterine carcinoma into the lumen of the tube become implanted on its mucosa, cancer cells escaping from an ovarian carcinoma into the peritoneal cavity might become implanted on the mucosa of the fimbriae and ampulla of the tube. The fimbriae of the tubes are immersed in the ascitic fluid

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IMPLANTATION CARCINOMA OF THE TUBAL MUCOSA SECONDARY TO CARCINOMA OF THE OVARY *

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It is the purpose of this paper to demonstrate changes in the mucosa of the fimbriae and ampulla of the Fallopian tubes of patients with ovarian carcinoma which may provide a fertile soil for the implantation and growth of cancer cells on its surface, and likewise to present the various stages in the actual implantation of cancer cells in these situations and the subsequent life history of the resulting secondary neoplasms. In addition I wish to emphasize that the pathogenesis, structure, form and life history of carcinomatous implants of ovarian origin on the tubal mucosa are the same as the pathogenesis, structure, form and life history of similar implants on the peritoneal serosa.

In 1934 the writer ¹ published the results of a study of a series of carcinomas of the tubes and ovaries secondary to carcinoma of the body of the uterus. In that paper 9 cases of carcinoma of the tubal mucosa were reported. In 2 of these cases the carcinoma evidently reached the tubal mucosa by lymphatic permeation or metastasis from the uterine neoplasm. In the remaining 7 cases, 1 of which had also been reported in an earlier paper,² the tubal carcinomas were judged to have arisen from cancer cells that had escaped from the uterine tumor through the uterine ostium of the tube into its lumen and had become grafted on the tubal mucosa.

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the absence of breaches in healthy epithelial surfaces — all these considerations should give pause to a too facile assumption of implantation metastasis, however plausible in explaining the conditions observed. Before adopting this explanation in any given case, it is essential to inquire whether the supposed implant tumours may not be either multiple primary growths or metastasis by other routes, lymphatic, transcoelomic or hemic.”

In discussing the origin of inoculation metastasis in the female genital tract Willis states: “Since the cavities of the healthy uterus and tubes are bacteria-free, and since they contain no lytic secretions and but little mucus, tumour implantation in the tubal mucosa or the endometrium must be regarded as by no means improbable. In the vagina or vulva, on the contrary, the conditions must be highly unfavourable to successful implantation. The literature contains many records of possible or probable examples of tumour inoculation in the female genital tract. In reviewing these it is important to recognize that in this territory local metastasis via lymphatic and venous channels is a frequent event, and that many seeming instances of implantation may equally well be attributed to spread by these familiar routes. In a brief but valuable paper, Glendining ⁴ (1910) advanced evidence for the occurrence of tubal mucosal implantation of tumor cells wafted by the fimbriae into the tubes from the peritoneal cavity, but the observations did not permit conclusive proof. Kaufmann (p. 1061) observed distension of the tube by carcinomatous masses secondary to a mucoid gastric cancer, and attributed these to mucosal implantation in the tube. Sitzenfrey (cited by Kaufmann), Kundrat ⁵ (1906), Werner ⁶ (1914), Sampson ² (1924), Offutt ⁷ (1932) and others have described specimens which strongly suggest retrograde passage of fragments of uterine cancer into the tubes with mucosal implantation in these organs.” However, none of the above mentioned authors described the various stages in the implantation of these cells on the tubal mucosa, which is so essential for the acceptance of this theory.

In conjunction with one phase of the problem of the present paper Glendining's ⁴ contribution is a valuable one. He reported the results of a careful study of the tubes from two patients with carcinoma of the ovaries secondary to carcinoma of the stomach. In both instances he found cancer cells in the lumens of the tubes

frequently encountered in patients with ovarian carcinoma. Cancer cells are usually present in this fluid. We have abundant evidence that cancer cells floating about in ascitic fluid and in the lumens of lymph vessels and of the tubes may not only be alive but may live a long time and actually increase in numbers in these situations. It is natural to assume, therefore, that these cells sometimes might become implanted on the surface of the fimbrial mucosa and also on the surface of other portions of the tubal mucosa, just as they become implanted on the lining of lymph vessels and on the surface of the peritoneum when a fertile soil arises for their implantation and growth.

In the study of the life history of any secondary neoplasm we should realize that its growth and spread, irrespective of its pathogenesis, depend on its inherent traits and likewise on the "soil" and the structure of the organ in which it is located. We must anticipate that if carcinoma becomes implanted on the tubal mucosa it may invade the mucosal lymph vessels and in this way it may present histological findings that may be confused with those resulting from metastases to the tubal mucosa from the ovarian carcinoma by way of the lymphatics. A knowledge of the lymphatics of the tubal mucosa, therefore, is essential for the study of the life history of all carcinomas of this mucosa. In order to be able to study intelligently implantation carcinoma of the tubal mucosa secondary to ovarian carcinoma, it is necessary to appreciate the changes in this mucosa caused by carcinoma reaching it by other ways than implantation.

THE STATUS OF THE IMPLANTATION OF CANCER CELLS ON THE SURFACE OF THE TUBAL MUCOSA

The origin of metastases from the implantation of cancer cells on epithelial surfaces has been and still is a debatable one. This subject is well presented by Willis³ in his excellent monograph published in 1934. He states: "There are strong *prima facie* reasons why we must regard inoculation metastasis as an improbable event in most situations. The presence of a rich bacterial flora in skin and in alimentary and respiratory mucous membranes, the presence of digestive enzymes and mucus in the alimentary tract and of mucus in the respiratory tract, the certain enfeebled vitality of fragments of tumour detached from ulcerating growths, and

hand, implants were not found in the peritoneal cavity of control animals injected only with cancer cells. Similar results were obtained by these workers when pieces of small glass rods and dead cancer cells were introduced into the peritoneal cavity of these animals prior to the injection of a suspension of the living cancer cells. They demonstrated that the localization of the peritoneal metastases was determined by the proliferation of the submesothelial connective tissue at the site of injury, which formed a supporting stroma for the living tissue cells subsequently introduced into the peritoneal cavity. They believed that dead cancer cells in contact with the serosa of human beings may cause changes favorable for the lodgement and growth of living cancer cells.

It was shown in a paper on implantation peritoneal carcinomatosis of ovarian origin, published by the writer⁹ in 1931, that the implantation of the cancer cell is made possible by injury to the peritoneum causing a local reaction of its submesothelial tissues with a disappearance of the mesothelium over this area. The degree of this reaction varied greatly in individual cases. In some instances it was slight. In others strands or masses of granulation tissue arose on the surface of the serosa, forming sessile or pedunculated outgrowths of this newly formed tissue. Cancer cells were frequently found anchored to the peritoneum by fibrin at the site of injury and attached to or enmeshed in the strands and polypoid outgrowths of granulation tissue. On the other hand, similar peritoneal reactions without cancer cells attached to or enmeshed in them were occasionally seen, especially in the early cases of peritoneal carcinomatosis. It is evident that these areas of newly formed tissue, whether slight or exuberant, supply the soil on or in which cancer cells floating in the peritoneal cavity lodge and grow. I do not know the cause of the injury nor why it is local. Obviously cancer cells escaping into the peritoneal cavity in some way are responsible.

The form of the mature implant varies with the type of the initial reaction of the peritoneum to the injury, the attempted repair of this injury, the growth of the cancer cells and the age of the implant. As a result of these processes carcinoma becomes embedded in peritoneal scars, encapsulated on its surface, enmeshed in adhesions and in the organized polypoid outgrowths of granulation tissue or, like a surgical skin graft, grows on the sur-

and carcinoma in the mucosal lymphatics. He believed that cancer cells floating in the peritoneal cavity were swept into the lumens of the tubes by their fimbriae, became grafted in their mucosa and from there penetrated the subepithelial lymphatics and thus escaped into the lymph vessels of the mesosalpinx. In both instances the efferent lymph vessels of the ovaries were permeated by the growth. Glendining correctly concludes that one cannot entirely exclude the possibility of the origin of the tubal carcinoma from the ovarian tumors by lymphatic permeation.

I have studied the microphotographs published by Glendining⁴ and believe that the carcinoma in the mucosal lymphatics in his cases probably came from the ovarian tumor by way of these vessels and not from the implantation of cancer cells on the tubal mucosa. I also believe that some of the clumps of cancer cells in the lumens of the tubes well may have reached this situation by growing out from the mucosal lymphatics through the overlying epithelium. This latter phenomenon I have frequently observed.

Even though Glendining's⁴ interpretation of the sequence of events in the specimens studied by him may not be correct, nevertheless he has contributed a very interesting and stimulating theory, namely, that cancer cells floating about in the peritoneal cavity may be swept by the fimbriae into the lumen of the tube, become implanted on the tubal mucosa, and by their invasion of the underlying lymphatics gain access to the lymphatic circulation of their host.

THE IMPLANTATION OF CANCER CELLS ON THE PERITONEUM AS A GUIDE IN THE STUDY OF THE IMPLANTATION OF SIMILAR CELLS ON THE TUBAL MUCOSA

I have assumed in the study of the present problem, until proved otherwise, that the pathogenesis of the implantation of cancer cells on the tubal mucosa should not differ greatly from the pathogenesis of the implantation of similar cells on the peritoneum.

In 1914, Jones and Rous⁸ published the results of a series of experiments in which Kieselguhr (finely ground diatomaceous earth) suspended in Ringer's solution was injected into the peritoneal cavity of mice, followed later by a suspension of mouse carcinoma cells. Implantations of carcinoma occurred at the site of the peritoneal injury caused by the Kieselguhr. On the other

lymph vessels as well as newly formed blood vessels participate in the development of cancer-free granulation tissue arising on the surface of the peritoneum of patients with peritoneal carcinomatosis. This tissue forms the stroma of peritoneal implants. Newly formed lymph vessels were found also in the stroma of some of these implants. In a few instances conditions were encountered suggesting that the presence of these newly formed lymph vessels in the stroma of an implant might have permitted an earlier spread of the neoplasm into the lymphatic circulation of its host than would have occurred had these vessels not been present (see Figs. 86, 111, 112 and 113 of that paper ¹⁰).

Since newly formed lymph vessels are sometimes present in newly formed tissue arising on the surface of the serosa of patients with peritoneal carcinomatosis, one would expect that carcinoma in preexisting lymphatics beneath the newly formed tissue might invade the newly formed lymph vessels. This condition has been encountered (see Figs. 129, 130 and 131 of previous paper ¹⁰).

If cancer cells become implanted on the tubal mucosa one would expect that they might have a life history similar to cancer cells implanted on the serosa and that some or even all of the phenomena just described might be encountered in the study of mucosal implants.

THE LYMPHATICS OF THE MUCOSA OF THE FIMBRIAE AND AMPULLA OF THE TUBE

In the study of carcinoma of the tubal mucosa secondary to carcinoma of the ovary I have been impressed with the great frequency of the location of these tumors in the distal portion of the tube and also with the presence of the growth in spaces in its mucosa which I believed might be lymph vessels. It appeared to me that in some instances the carcinomas in the mucosal lymphatics had not necessarily come from the primary ovarian growth through these channels but well may have reached their present situation from the spread of a secondary tumor of the tubal mucosa of other origin than lymphatic metastasis or permeation from the ovarian tumor. Irrespective of the method of origin of those secondary tumors from the ovarian growth, a knowledge of the lymphatic circulation of the mucosa of the tube, especially that of its distal portion, is essential in order to understand the patho-

face of the peritoneum without encapsulation. All stages in the development of these various types of peritoneal implants have been described by the writer.⁹ We must, therefore, regard an *implant as a true neoplasm which may consist solely of cancer cells, but more often consists of both cancer cells and a stroma of newly formed tissue which is derived from its host.*

The implantation of cancer cells on the tubal mucosa cannot be accepted until all stages in the development of the various types of these secondary tumors in this situation have been demonstrated. Whether or not the various stages in the implantation of cancer cells in the two situations are the same will be shown.

THE LIFE HISTORY OF THE PERITONEAL IMPLANTS AS A GUIDE IN THE STUDY OF THE LIFE HISTORY OF MUCOSAL IMPLANTS OF THE FALLOPIAN TUBE

What happens to cancer cells implanted on the peritoneum? Some die, others apparently multiply very slowly and remain efficiently encapsulated by dense connective tissue for a long time. This latter phenomenon is very evident in some of the mature pedunculated polypoid implants. If the cancer cells of the implant are not encapsulated an opportunity for the dissemination of some of these cells from this focus into the peritoneal cavity is present.

We realize that many patients with peritoneal carcinomatosis do not die from the primary ovarian tumors but from intestinal obstruction caused by the invasion of the intestines by the carcinoma implanted on their surfaces. This is an indication of the marked invasive trait of the cancer cells in some of these implants. They invade the tissues of their host like a primary tumor. Carcinoma may be found in the lymphatics beyond the advancing margin of the growth (see Figs. 51 and 55 of previous paper⁹).

If cancer cells become implanted on the tubal mucosa we would expect that they might have a life history similar to cancer cells implanted on the serosa and that they might invade the tubal lymphatics and even reach the ovary through these channels. The latter phenomenon (which is not impossible) would cause a confusing picture.

In a recent paper by the writer¹⁰ on the origin and significance of newly formed lymph vessels in carcinomatous peritoneal implants, it was demonstrated that in some instances newly formed

ampulla. I found that the pattern of the lymphatics in the two situations was the same.

For descriptive purposes the lymphatics of the ampullar and fimbrial mucosa may be divided into two plexuses; one situated in the mucosa at the base of and between the folds, and the other in the folds. Vessels from the plexus in the folds empty into the plexus at the base of the folds. Thus the lymphatics of one mucosal fold are united with those of adjacent folds. This general pattern prevails in all sizes and types of mucosal folds whether in the ampulla or fimbriae (see Figs. 2, 3 and 5). Since the lymphatics of the ampullar and fimbrial mucosa are true capillaries without valves a free circulation of the lymph is assured in all directions in the plexuses.

I was able to demonstrate that the lymphatics at the base of and between the mucosal folds of the fimbriae about the ostium of the tube are continuous with similar lymphatics of the distal portions of the ampulla. Also when a fold of fimbrial mucosa is a continuation of a longitudinal fold of the ampulla the lymphatics in the two folds as well as at their bases are continuous. This well may account for the frequent presence of carcinoma both in the lymphatics of the fimbrial mucosa and in those of the adjacent ampullar mucosa of the same tube.

Evidence was found indicating that the mucosal lymphatics of the fimbriae drain into vessels in the wall of the infundibulum and also into vessels in the mesosalpinx beneath the ovarian fimbriae just as the lymphatics of the ampullar mucosa drain into vessels penetrating the wall of the tube beneath it.

I was unable to detect any evidence of an anastomosis between the lymphatics of the fimbrial mucosa and the subserosal lymph vessels at the mucoserosal junction even in fimbriae in which the mucosal lymph vessels were filled with carcinoma. Andersen ¹² has shown that the subserous lymph capillaries of the distal portion of the ampulla of the sow's tube are few and inconspicuous as compared with the rich lymphatic plexus of the mucosa. It is my impression that the same is true in the tubes of human beings.

An anastomosis between lymph vessels coming from the hilum of the tubal pole of the ovary and the lymphatics of the adjacent ovarian fimbriae may well exist but was not positively demonstrated by me. The suggestion of the possibility of such an

genesis of some of these tumors and the possible subsequent life history of all of them.

In endeavoring to ascertain the significance of the various conditions encountered in the study of these secondary carcinomas of the tubal mucosa, I found that I was greatly handicapped by a lack of any conception of the lymphatic circulation of the mucosa of the tube. I therefore attempted to ascertain the distribution of the lymph vessels in the mucosa of the fimbriae of the tube and their relation to the lymphatics of the ampulla and to those of the mesosalpinx and of the ovary. Some of the results of these endeavors have recently been published.¹¹

I have been unable to find in the literature an adequate description of the pattern of the lymphatics in the mucosa of the ampulla and fimbriae of the tube in human beings. Andersen,¹² however, has given an excellent description of the injected lymphatics of all layers of the ampulla of the tube of the sow. Owing to technical difficulties she was unable to inject the lymphatics of the mucosa of the infundibulum.

I have observed both in the mucosa and in the walls of the tubes from patients with carcinoma of either the uterus or the ovary, spaces that were filled with carcinoma as with an injection mass (see Figs. 10 and 11). I have also frequently seen irregular empty spaces in other portions of the tubal mucosa just described and also in the mucosa of many normal tubes which, in form and in situation, resembled the spaces filled with carcinoma. In these empty spaces an endothelium-like lining could be detected. I inferred that these carcinoma-filled and empty spaces were lymphatics. I gathered this material and compared these spaces with Andersen's ¹² illustrations and descriptions of the injected lymphatics of the mucosa of the ampulla of the sow. I found that the judged lymphatics observed by me were similar, both in form and in distribution, to the injected lymphatics of the sow's tube shown by Andersen.

Since the mucosa of the fimbriae is a continuation of the mucosa of the ampulla through the abdominal ostium of the tube and has the same histological structure as the latter, the distribution of the lymphatics in these two situations should be similar. I studied carcinoma-filled and empty lymphatics of the fimbrial mucosa by comparing them with similar lymphatics in the mucosa of the

and carcinomatous implants occur on the fimbrial and ampullar mucosa which has a richer and possibly more accessible lymphatic plexus than the ampullar serosa, one would anticipate that some or all of the phenomena just described might be found in the study of mucosal newly formed tissue and implants.

MATERIAL AND METHODS OF STUDY

The material for this study was obtained from the gynecological service of the Albany Hospital. The technical part of the work was carried on in the pathological laboratory of that hospital and the Albany Medical College. On the other hand, the greater part of the time spent by me in the actual study of the problem, including the microscopic examination of sections and writing, was done during "vacations" away from routine professional responsibilities.

From 1921 to 1936, inclusive, 148 patients with ovarian carcinoma were operated upon in the gynecological service of the Albany Hospital. Judged secondary carcinoma of 1 or both tubes was encountered in 23 of these patients. Since carcinoma was found in both tubes in 9 cases, 32 tubes with secondary carcinoma of their mucosa, multiple in many instances, were available for this study.

During the earlier and major part of this period I was greatly interested in peritoneal carcinomatosis. After the removal of the uterus, tubes and ovaries from patients with ovarian carcinoma, the tubes with serosal implants were severed from the ovary before blocking them. Thus an opportunity to study critically the possible extension of the ovarian carcinoma into the lymph vessels of the mesosalpinx was lost in these cases. During that period the only portions of the tubes that were carefully examined microscopically were those with obvious carcinomatous implants on their serosa. The fimbriae were not sectioned unless they grossly presented the appearance of malignancy. If the tubes appeared normal usually only one block was cut from each tube. From the standpoint of the present problem this method of studying the greater portion of this material was incomplete and gives no statistical data in determining the frequency of implants on the tubal mucosa.

However, in recent years special attention has been paid to the

anastomosis was found in only two of my specimens. Polano¹³ found a lymphatic loop in the ovarian fimbriae by injecting the lymphatics of the ovary. Kroemer,¹⁴ by injecting the tubal lymphatics, found communicating branches running along the ovarian fimbriae.

Andersen demonstrated that in the sow (Fig. 1 of her paper¹²) the lymph vessels from all portions of the tube, including the fimbriae, converge to form the subovarian lymph plexus which also receives lymph vessels from the ovary and the uterus. She refers in her paper to the contributions of Poirier, Bruhns, Bartels, and Poirier and Cuneo, indicating that lymph vessels from the tube unite with similar vessels from the ovary and uterus.

Pellé and Pellé¹⁵ (1931) by first injecting the lymphatics of the tubes with Paris blue and then those of the ovary or uterus with cadmium yellow observed that the blue colored lymph vessels coming from the tubes soon became green, thus indicating an early communication between the lymph vessels of the tube and those of the ovary and uterus. The anatomical observations just mentioned may be further confirmed by the abundant evidence in pathological studies that carcinoma may spread from either the uterus or the ovary to the tube, by way of the lymph vessels (see Figs. 10 and 11).

A study of the lymphatics of the fimbrial and ampullar mucosa indicates that they are very abundant and that while the larger ones are usually more centrally situated in the mucosal folds than the blood vessels, a very superficial, almost subepithelial, portion of the mucosal plexus may sometimes be detected (see Figs. 7 and 8). The mucosal lymphatics of the ampulla apparently are larger, more abundant, and possibly nearer the surface than the subserosal lymphatics.

The following conditions have been demonstrated in previous papers by the writer: the invasion of the lymphatics of the tubal wall by carcinoma implanted on its serosal surface; the presence of newly formed lymph vessels in cancer-free granulation tissue arising on the serosa of the ampulla of the tubes of patients with peritoneal carcinomatosis; the extension of carcinoma in the lymphatics of the tube into the lymph vessels of newly formed tissue on its serosa, and the presence of newly formed lymph vessels in the stroma of serosal tubal implants. If granulation tissue

from the same or different tubes. The main object in publishing a large number of microphotographs is that others may study them as I have, form their own conclusions and compare this material with their own. Since I desire to demonstrate that the pathogenesis, structure, form and life history of carcinomatous implants on the tubal mucosa are the same as the pathogenesis, structure, form and life history of similar implants on the peritoneum, I have chosen to publish microphotographs of serosal implants present in some of these cases. This important feature has increased the number of illustrations.

CASE REPORTS

The following 13 cases have been selected from the 23 of carcinoma of the tubal mucosa secondary to carcinoma of the ovary in order not only to report briefly the most important cases with implants on the tubal mucosa, but also, for the sake of comparison, to include 4 others in which the secondary mucosal carcinomas were obviously of other origin than implantation. The latter cases are presented first. The main purpose of the report of these 13 cases is to supplement the illustrations and their legends, which constitute the most important part of this paper since they present my observations and interpretations better than any written description alone.

CASE 1. Carcinoma of the ampullar and fimbrial mucosa of the left tube secondary to adenocarcinoma of the left ovary, which had invaded the mesosalpinx, including its lymph vessels, and thus may have gained access to the tubal lymphatics. The patient, A. H. No. 93646, nulliparous and aged 46 years, was operated upon Nov. 9, 1923. A large tumor of the left ovary was found, which was wedged in the pelvis and extended upward nearly to the level of the umbilicus. There was no gross evidence of peritoneal carcinomatosis. The right tube and ovary appeared normal. Both tubes and ovaries and the uterus were removed. The patient survived the operation but ultimately died of carcinoma. The evidence, found in the study of this specimen, indicates that the carcinoma of the tubal mucosa arose from the ovarian tumor through the lymph vessels (see Figs. 11 to 14 inclusive). Carcinoma was found in the right tube only in the form of serosal implants and in the right ovary in the form of lymphatic permeations

fimbriae of the tubes of patients with ovarian carcinoma. They are inspected at the time of the operation. Great care is exercised that they shall not be traumatized during the removal of the pelvic organs. Should they appear abnormal, careful drawings are made of them. All are studied microscopically.

All surgical specimens are described in the pathological laboratory, tissue is removed, fixed in Zenker's solution, embedded in paraffin, cut, stained and examined as a routine procedure. This routine examination of surgical specimens is of greatest value for records and diagnostic purposes but is not sufficient for the intensive study of special problems. Through the courtesy of Dr. Victor C. Jacobsen, the former director of the laboratory, and of Dr. Arthur W. Wright, the present director, I have retained specimens or portions of specimens in which I have been specially interested, saving for them sufficient material for routine laboratory records.

All of the tissue from which the sections shown in this paper were made was fixed in 10 per cent formalin and embedded in celloidin. This procedure lessens unequal tissue shrinkage. As a result fewer distortions and exaggerated tissue spaces occur than in paraffin embedding after formalin fixation. The sections were stained with hematoxylin and eosin.

I block the tissue myself. The embedding of this tissue, section cutting and staining are entrusted to a well trained technician who studies the gross specimen with me when the blocks are made and understands how the blocks are to be mounted and what to look for in each block.

Serial sections have been extensively employed in recent years and are sometimes indispensable. In some instances all sections of a portion of a block are stained and studied but more often sections are stained and mounted at intervals, the latter depending on the judged importance of the tissue in a given instance. All sections, however, are saved for further study if this is indicated. Frequently a section is stained and examined while trimming the block in order to ascertain if serial sections are indicated. Since complete serial sections are time consuming and frequently unfruitful they are not made unless they are indicated by a test section.

Microphotographs have been extensively used in the study of this and other problems. I find that they are of the greatest value in the comparative study of conditions present in different sections

the left ovary and a peritoneal carcinomatosis including metastases to the omentum were found. The opposite tube and ovary appeared normal. Both tubes and ovaries and the uterus were removed and a portion of the omentum was resected. The patient recovered from the operation but eventually died of carcinoma. The carcinoma of the ovary had invaded the fimbriae of the left tube (see Figs. 21 and 22). Carcinoma was not found in the right tube and ovary.

CASE 5. Multiple papillary carcinoma of the tubal mucosa secondary to carcinoma of the ovary, due to the implantation of cancer cells on the mucosa of the tube which primarily had escaped from the carcinoma of the ovarian cyst into the lumen of the tube through its patent ostium (tubo-ovarian cyst): The patient, A. H. No. 2960-31, nulliparous, aged 50 years and 2 years past the menopause, had had the appendix and right tube and ovary removed at the age of 20 years. At the second operation, April 24, 1931, a tubo-ovarian cyst on the left side was found. Peritoneal carcinomatosis was not evident. The left tube and ovary and the uterus were removed. The patient recovered from the operation but eventually died of carcinoma.

The specimen was hardened in formalin before incising the tubo-ovarian cyst (see Fig. 23). The series of sections of the tube in this case are sufficiently complete to enable one to study all stages in the pathogenesis of the implants on the tubal mucosa.

The following observations were made in the study of this tubo-ovarian cyst:

1. Changes in the tubal mucosa favorable for the implantation of cancer cells which had escaped into the lumen of the tube from the carcinomas of the ovarian cyst and which were shown also to have escaped from the secondary carcinomas of the tubal mucosa. These changes in the tubal mucosa are: (a) sessile and pedunculated polypoid outgrowths of granulation tissue on the surface of the tubal mucosa (see Fig. 27); and (b) local reactions of the subepithelial tissues of the mucosa, manifested by a proliferation of the tissue cells and associated with either a partial or a complete loss of the overlying epithelium.

2. Cancer cells attached to and becoming embedded in granulation tissue on the tubal mucosa (see Figs. 28 and 30). Cancer cells, splinted by fibrin, on the areas of the mucosa with a local

and emboli. The portal of entry of the carcinoma in the lymph vessels of the right ovary was not determined.

CASE 2. Carcinoma of the mucosa of the left tube including its fimbriae, secondary to adenocarcinoma of the left ovary, with evidence that the tubal carcinoma was derived from the ovarian tumor through the lymph vessels. The patient, A. H. No. 6447-29, parous and aged 56 years, was operated upon Sept. 16, 1929. The uterus contained multiple leiomyomas. A tumor, about 8 cm. in diameter, had replaced the left ovary. The right ovary and tube appeared normal. Metastases were present in the posterior cul-de-sac but none were detected in the omentum. Both tubes and ovaries and the uterus were removed. The patient survived the operation but eventually died of carcinoma. Carcinoma can be seen extending from the ovarian tumor in lymph vessels which accompany the blood vessels in the hilum of the ovary (Fig. 15) and also in the lymph vessels of the mesosalpinx and tubal wall. The carcinoma of the tubal mucosa, including its fimbriae, evidently arose from this source (see Figs. 16 and 17).

CASE 3. Carcinoma of the mucosa of the uterine portion of the left tube and also of its isthmus and ampulla secondary to an advanced adenocarcinoma of the ovary which had invaded the left cornu of the uterus and the proximal portion of the tube. The patient, A. H. No. 5466-34, parous and aged 56 years, was operated upon July 20, 1934. Bilateral ovarian carcinoma and an extensive peritoneal carcinomatosis including metastases to the omentum were found. The uterus contained multiple leiomyomas. The distal ends of both tubes appeared normal. The ovarian tumors were adherent to the uterus and the proximal portion of both tubes. Both tubes and ovaries and the uterus were removed. The patient survived the operation but eventually died of carcinoma. The left tube and uterine cornu with the portion of the ovarian tumor attached to them were carefully studied. By continuous extension the ovarian carcinoma had invaded the left uterine cornu and the proximal portion of the tube including its mesosalpinx (see Figs. 18 to 20 inclusive).

CASE 4. Carcinoma of the fimbriae of the left tube secondary to adenocarcinoma of the ovary, due to continuous extension of the primary ovarian growth. The patient, A. H. No. 56392, nulliparous and aged 52, was operated upon Sept. 20, 1922. A carcinoma of

were observed (see Figs. 47 and 54). In the second type carcinoma has replaced the epithelium of portions of the mucosal folds of the ampulla and presents the appearance of having been grafted in that epithelium (see Figs. 55 to 58 inclusive). The early stages in the pathogenesis of this latter type of implant, so well shown in the previous case, were not detected in the study of the tubes from the present case.

CASE 7. Carcinoma of the mucosa of the fimbriae of both tubes secondary to adenocarcinoma of the ovaries, due to the implantation of cancer cells in granulation tissue arising on the surface of the fimbrial mucosa. The patient, A. H. No. 3679-35, nulliparous and aged 41 years, was operated upon April 27, 1935. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis, including metastases to the omentum, were found. Both tubes and ovaries and the uterus were removed (see Fig. 59). The patient survived the operation and had deep X-rays, but eventually died of carcinoma. Three implants, of the polypoid type, were found attached to the fimbrial mucosa of the right tube (see Figs. 63 and 64). Two of these implants are mature (see Figs. 66 and 68). The third implant presents an early stage in the development of this type of implant, namely, the embedding of cancer cells in the tuft of a pedunculated polypoid outgrowth of granulation tissue which had arisen on the surface of a mucosal fold (see Figs. 65 and 67). Only one implant was present on the mucosa of the fimbriae of the right tube. It is mature and of the pedunculated polypoid type (see Figs. 69 and 70). Newly formed lymph vessels are present in its pedicle in close proximity to the carcinoma of the body of the implant (see Fig. 71). Carcinoma was not found in the mucosa of the ampulla nor in the lymph vessels of any portion of the tubes. The histological structure of these mucosal implants is the same as the structure of similar serosal implants from the same patient shown in Figures 50, 51, 52, 58 and 60 of a previous paper.¹⁰

CASE 8. Multiple carcinomas of the mucosa of both tubes, including their fimbriae, secondary to adenocarcinoma of both ovaries. The carcinomas of the right tube arose from a continuous extension of the ovarian growth invading the fimbriae and also from the implantation of cancer cells on the mucosa of the fimbriae and the ampulla. The carcinomas of the left tube arose from lymphatic metastasis and permeation from the ovarian growth,

reaction of the subepithelial tissues and a loss of the overlying epithelium (see Figs. 35, 36 and 37).

3. Neoplasms consisting of carcinoma embedded in or growing on the surface of polypoid newly formed tissue which had arisen on the surface of the tubal mucosa (see Figs. 29 and 31). Carcinoma replacing portions of the tubal epithelium as though grafted in it (see Figs. 32, 33, 34 and 35).

4. All stages in the development and growth of these types of mucosal implantation metastases, which are demonstrated in Figures 28 to 41 inclusive.

It is evident, therefore, that the pathogenesis, structure and form of the carcinomatous implants on the tubal mucosa in this case are the same as the pathogenesis, structure and form of similar implants on the serosa of patients with peritoneal carcinomatosis. The ovary was not studied as thoroughly as the tube. However, it appears that the pathogenesis of the judged secondary carcinomas of the ovarian cyst is the same as the pathogenesis of the carcinomas of the tubal mucosa.

CASE 6. Carcinoma of the fimbrial mucosa of both tubes secondary to adenocarcinoma of the ovaries, due to the continuous extension of the primary tumors to the fimbriae with replacement of portions of their mucosa. Multiple carcinomas of the mucosa of the fimbriae of the left tube due to the implantation of cancer cells on its surface. Multiple carcinomas of the mucosa of the ampulla of the right tube due to the implantation of cancer cells on its surface, which were disseminated into the lumen of that tube from the portion of the ovarian growth that had replaced the fimbriae and had extended through the abdominal ostium of the tube into the lumen of the ampulla. The patient, A. H. No. 7062-32, nulliparous and aged 73 years, was operated upon Oct. 5, 1932. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum were found. Both tubes and ovaries and the uterus and an epiploical appendage were removed. The patient survived the operation, but eventually died of carcinoma.

Two types of mucosal implants were found in this case. One type consists of polypoid newly formed tissue, which had arisen on the surface of the fimbrial and ampullar mucosa, with carcinoma embedded in it or growing on its surface (see Figs. 46, 47, 53 and 54). Various stages in the pathogenesis of these polypoid implants

plants on the serosa of the tubes were similar to those on the appendage. All stages in their development could also be seen in this situation. However, carcinoma was found in the lymph vessels beneath some of the larger serosal implants on the right tube. It was judged that the carcinoma in these lymph vessels might have come from the spread of the carcinoma in the implants above them rather than primarily from the ovarian growth, even though the carcinoma in the ovary had invaded the lymph vessels of that organ (see Figs. 85 to 95 inclusive of a previous paper,¹⁰ and also Fig. 101 of the present paper).

Longitudinal sections of the fimbriae of the right tube present an interesting and difficult problem. The lymphatic vessels of the fimbrial folds at the right in Figure 102 are filled with carcinoma, as with an injection mass evidently coming from the extension of the growth in lymph vessels in the base of these folds and between them. Carcinoma is not present in the lymph vessels of the fimbrial mucosa at the left. The tips of the mucosal folds in the center of this section are fused together by newly formed tissue of various ages containing carcinoma. On the other hand, the lymph vessels in the base and between these fused folds are not distended with carcinoma as in the folds at the right. A conglomerate tumor consisting of fused polypoid outgrowths of newly formed tissue of various ages containing carcinoma is attached by a short pedicle to the fused portion of the folds. It is judged that this conglomerate tumor has arisen from the fusion of polypoid implants (see Fig. 107 and its legend). The picture here is similar if not identical with the cluster of implants on the serosa of the same tube shown in Figure 101.

To the right of the base of the conglomerate tumor, just described (Fig. 107), is a similar tumor consisting of the tips of mucosal folds which are united by newly formed tissue containing carcinoma. Very early granulation tissue "f," with small clumps of cancer cells embedded in it, apparently is of the same age as outgrowth "a." Beneath this tissue is older newly formed tissue containing a larger amount of carcinoma. The latter tissue has arisen from adjacent mucosal folds. The conditions shown here easily could have resulted from the fusion of newly formed tissue similar to that arising from the mucosal fold in Figure 106. Cancer cells "g" are shown becoming implanted on the tip of newly formed

and also from the implantation of cancer cells in granulation tissue arising on the fimbrial mucosa. The patient, A. H. No. 9895, parous and aged 47 years, was operated upon Sept. 21, 1935. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum and the umbilicus were found. Both tubes and ovaries and the uterus were removed and the umbilicus was excised. The patient had deep X-ray treatments following the operation, but eventually died of carcinoma. Three secondary carcinomas of the right tube were found, one due to an extension of the ovarian carcinoma invading the ovarian fimbriae (see Fig. 85), the second a polypoid implant on the mucosa of the fimbriae (see Fig. 86), and the third a judged early implant on the mucosa of the ampulla (see Fig. 77). Carcinoma was not found in the lymph vessels of this tube. The carcinomas of the fimbriae of the left tube arose both from lymphatic metastasis and permeation from the ovarian carcinoma (see Figs. 87 to 91 inclusive), and also from the implantation of cancer cells in granulation tissue which had developed on the surface of its mucosa (see Fig. 93). The carcinoma of the ampullar mucosa of this tube (Fig. 92), arose from lymphatic metastasis and permeation. For a further consideration of the findings in this case see Figures 73 to 96 inclusive.

CASE 9. Carcinoma of both the fimbrial and the ampullar mucosa of the right tube and of the ampullar mucosa of the left tube secondary to carcinoma of both ovaries. The secondary tubal carcinomas in all three situations were judged to be of probable implantation origin with subsequent invasion of the mucosal lymph vessels. The patient, A. H. No. 7467-30, parous and aged 42 years, was operated upon Oct. 15, 1930. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum were found. Both tubes and ovaries, the uterus and an epiploic appendage were removed. The patient recovered from the operation but eventually died of carcinoma. Many peritoneal implants from this patient have been studied and some of these have been described in two previous papers ^{9, 10} (see Case 3 and Case 5 of these papers). The implants on the epiploic appendage were especially instructive. They were of various types and showed all stages in their development. Carcinoma was not found, in spite of a careful search, in the lymph vessels of the appendage. The im-

of the tubal epithelium but may have invaded the mucosal lymph vessels (see Figs. 110 to 116 inclusive). In all instances where carcinoma is present in lymph vessels beneath either a serosal or a mucosal metastasis resembling an implant, both the source of the carcinoma in the lymph vessels and the pathogenesis of the metastasis are debatable. If carcinoma implanted on the tubal serosa sometimes invades the underlying lymph vessels it is rational to believe that carcinoma implanted on the tubal mucosa sometimes might invade the mucosal lymph vessels, especially since these lymph vessels are more abundant and possibly more accessible than those of the serosa.

CASE 10. Carcinoma of the mucosa of the ampulla of the tube secondary to carcinoma of the ovary, probably due to the implantation of cancer cells in granulation tissue arising on the surface of the mucosa. The patient, A. H. No. 92806, nulliparous and aged 41 years, was operated upon Sept. 29, 1923. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum were found. Both tubes and ovaries and the uterus were removed. The patient survived the operation, but eventually died of carcinoma. The ovarian carcinomas were small and apparently arose from the surface epithelium of the ovaries. The pathogenesis of the peritoneal implants in this case have been described (in Case 4 of a previous paper⁹). Two metastases were found attached to the mucosa of one tube. They are almost exact duplicates of the polypoid implants present on the serosa of the opposite tube (see Figs. 117 to 120 inclusive). Carcinoma was not found in the lymph vessels of either the ovaries or the tubes but was present in the lumens of both tubes, the fimbriae of which appeared normal. In a few situations carcinoma was found in the lymph vessels beneath some of the implants on the parietal peritoneum and also beneath the implant attached to the serosa of the mesosalpinx shown in Figure 121. Circumstantial evidence indicates that the metastases in the mucosa of the tube arose from the embedding of cancer cells, present in the lumen of the tube, in granulation tissue which had developed on the surface of the tubal mucosa just as similar implantations arise on the surface of the serosa.

CASE 11. Carcinoma of the mucosa of the fimbriae and of the ampulla of the right tube secondary to carcinoma of the ovary

tissue arising from the fimbrial mucosa. This is a miniature repetition of the process shown in Figure 106. I am unable to decide whether the structure marked "e" is a mature polypoid implant or the tip of a fold infiltrated with carcinoma. Its origin was missed in the incomplete series of sections. A cross section of a mucosal fold "h" (easily recognized as such on careful study under higher magnification) shows carcinoma in a central large lymph vessel which apparently is continuous with the growth in the judged smaller peripheral lymph vessels of the fold. Beneath this is another portion of the same fold which is infiltrated with carcinoma, some of which is in lymph vessels. It is impossible to determine whether the picture shown in this field represents the invasion of the folds, including their lymph vessels, by carcinoma implanted in newly formed tissue on their surfaces (see arrows) and the subsequent extension of the growth in lymph vessels, or the spread of carcinoma in lymph vessels into the tissues of the folds and thence into the newly formed tissue arising on the folds.

We have positively demonstrated in the study of these conglomerate tumors in this and other sections of this series that cancer cells have become implanted in granulation tissue arising on the mucosal folds of the fimbriae of this tube and that when this tissue arises on adjacent folds these newly formed tissues become fused. We have also shown that the implanted cancer cells have grown in these newly formed tissues as in similar tissues arising both on the serosa and on the mucosa of other cases. We may infer that the carcinoma in the older judged implants has invaded the folds beneath them and even may have penetrated the lymphatics of these folds. In this way the conditions found in the study of the fimbriae of this tube may be accounted for. However plausible this explanation appears to me I fully realize that it may not be conclusive in explaining the entire picture found in these fimbriae since the growth in the ovary had invaded the lymph vessels of that organ. Carcinoma in the ovarian lymph vessels might have spread to the lymph vessels of the fimbrial folds and from this source might have invaded the newly formed tissue arising on the folds in which cancer cells in the ascitic fluid became implanted.

Clumps of cancer cells similar to those present in the ascitic fluid are present in the lumens of both tubes (see Figs. 108, 109 and 110). In both tubes carcinoma has not only replaced portions

vessels of the distal portion of the mesosalpinx. I believe that this polypoid tumor on the fimbrial mucosa is an implant and that the carcinoma in it has invaded the underlying lymph vessels just as the carcinoma in similar implants on the serosa, from the same patient, have invaded the lymph vessels beneath them.

A large pedunculated polypoid mucosal metastasis is present in the ampulla of the right tube (see Fig. 144). This is similar to the preceding one and has grown by the attempted implantation on its surface of single clumps and collections of clumps of cancer cells which had migrated into the lumen of the ampulla of the tube through its abdominal ostium (see Figs. 137 and 143). The structure, growth and fate of this conglomerate tumor is fully described in the legend of Figure 144. In Figure 145 is shown the judged pedicle "a" of this polypoid tumor. The pedicle which appears in cross section consists of newly formed tissue containing blood vessels best seen under higher magnification. It has the characteristic structure of the pedicles of other mucosal polypoid implants shown in this paper (see Figs. 67 and 68). Although it was followed through several sections, its attachment to the tumor above it could not be detected (series of sections not complete). The small size of the blood vessels in this pedicle would easily account for the necrosis of a large portion of the tumor. Lymph vessels cannot be detected in the pedicle. However, they may be present since they occur in similar pedicles. Carcinoma was not found in any of the sections of this pedicle. Cancer cells might, at one time, have escaped from the tumor through lymph vessels in the pedicle into the lymph vessels of the mucosa from which the newly formed tissue of the tumor, including its pedicle, arose. In Figure 146 is shown a portion "a" of the attachment of the pedicle to a mucosal fold and carcinoma "b" in a lymph vessel of the mucosal fold in close proximity to the origin of the pedicle. An embolus of cancer cells "c" appears in a lymph vessel of a mucosal fold adjacent to the previous fold. The lymph vessels, in which the carcinoma is situated, are in adjacent secondary folds of the same primary mucosal fold. Therefore cancer cells might easily migrate from one secondary fold to the other. Carcinoma was not found in any other lymph vessels of the ampullar mucosa or in those of the tubal wall except beneath the attachment of serosal implant "g" of Figure 144 (see also Figs. 132 to 136 inclusive). Carcinoma

which well may have arisen from the implantation of cancer cells on the tubal mucosa. Carcinomas of the mucosa of the fimbriae and of the ampulla of the left tube which are so far advanced that it is impossible to determine the pathogenesis of the initial tubal tumor. The patient, A. H. No. 8617-33, parous and aged 53 years, was operated upon Nov. 27, 1933. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum were found. Both tubes and ovaries, the uterus and two epiploical appendages were removed. The patient survived the operation but eventually died of carcinoma. The study of the polypoid serosal implants is most instructive as a possible aid in determining the pathogenesis of the mucosal metastases of the right tube. Multiple polypoid implants are present on one epiploic appendage without any evidence of carcinoma in the preexisting tissues of the appendage (see Figs. 122 and 123). In another epiploic appendage, with similar but apparently older polypoid implants on its surface, carcinoma is present in the lymph vessels of the appendage with positive proof that it is continuous with the carcinoma in an implant which had invaded the tissues of the appendage beneath it and had thus penetrated the lymphatics (see Figs. 124 to 130 inclusive). Similar dual phenomena were observed in similar polypoid implants on the serosa of the right tube and mesosalpinx (see Figs. 131 to 136 inclusive). The metastasis of the fimbrial mucosa of the right tube (Fig. 138) consists of a pedunculated polypoid tumor of newly formed tissue, with carcinoma embedded in it, which is attached by a pedicle to a mucosal fold of the fimbriae. The histological structure of this metastasis is similar to that of the serosal implants just described. Carcinoma is present in the mucosal fold and in its lymph vessels beneath the attachment of the pedicle of the polypoid tumor to the fold. The carcinoma in the polypoid tumor is apparently continuous with the carcinoma of the mucosal fold (see Fig. 139). Recent newly formed tissue with cancer cells becoming embedded in it (see Figs. 138 and 142) has arisen on the surface of this polypoid tumor. This phenomenon frequently occurs in both serosal and judged mucosal implants and is one way in which these tumors increase in size. The carcinoma, in the lymph vessels of the fimbriae, occurred only in those of the mucosal fold beneath the polypoid tumor and of nearby folds. Carcinoma was not found in the lymph

pate that carcinoma implanted on the mucosa would gain access to the underlying lymph vessels more readily than when implanted on the serosa.

CASE 12. Carcinoma of the mucosa of the fimbriae of both tubes secondary to carcinoma of the right ovary. The carcinoma of the fimbriae of the right tube was probably due to lymphatic metastasis or permeation from the ovarian carcinoma. Some and possibly all of the carcinomas of the fimbriae of the left tube were due to implantation. The patient, A. H. No. 1130-26, parous and aged 49 years, was operated upon July 7, 1926. A large carcinomatous cyst of the right ovary and an extensive peritoneal carcinomatosis including metastases to the omentum were found. Both tubes and ovaries, the entire uterus, appendix and an epiploic appendage were removed. A portion of the omentum was excised. The patient recovered from the operation, was temporarily improved, but eventually died of carcinoma.

A hydrosalpinx was present on the right side with a portion of the ovarian fimbriae sticking out from its distended closed end like a sore thumb from a closed fist. The lymphatics of this portion of the fimbriae are filled with carcinoma as with an injection mass (see Fig. 151). Carcinoma was not found in any other portion of this tube.

Multiple carcinomatous implants are present on the surface of the left ovary; apparently one of these had invaded the ovary (see Fig. 152). The fimbriae of the left tube appeared swollen. Both serosal and mucosal implants can be seen on this portion of the tube (see Fig. 154). Carcinoma apparently arising in the fimbriae about the abdominal ostium of the tube has invaded the fimbrial and ampullar mucosa including the lymph vessels. For evidence that this carcinoma may primarily have been of implantation origin with subsequent invasion of the mucosal lymph vessels, see Figures 151 to 156 inclusive.

CASE 13. Carcinoma of the mucosa of the fimbriae and ampulla about the abdominal ostium of the left tube secondary to carcinoma of the ovary. Some and possibly all of the carcinomas of the fimbrial mucosa are of implantation origin. The patient, A. H. No. 856618, nulliparous and aged 53 years, was operated upon July 22, 1922. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum were found.

in a lymph vessel of the mesosalpinx was found in only one section. It appeared as an embolus in a lymph vessel of a portion of the mesosalpinx near the attachment of serosal implant "g" of Figure 144, and could easily have come from this source. Why should carcinoma be found only in lymph vessels of the ampullar mucosa in close proximity to the origin of the judged pedicle of the mucosal metastasis shown in Figure 144, unless the carcinoma in one of the two situations was primarily derived from the other? The histological structure of the nucleus of this mucosal metastasis is the same as the structure of serosal implants from the same patient without and with evidence of the spread of the carcinoma in the implants through their pedicles into the lymph vessels of their host. I believe that both the origin and the subsequent life history of the serosal and mucosal metastases just described are the same.

Two small carcinomas of the ampullar mucosa of the right tube were encountered which presented the appearance of having arisen either from the tubal epithelium or from the implantation of cancer cells. Many of these cells were floating about in the lumen of the tube. One of these tumors is shown in Figure 150. I believe that the pathogenesis of such an implant is demonstrated in Figures 148 and 149.

When carcinoma is found in lymph vessels in close proximity to a secondary carcinoma one must always consider the possibility of its origin from the primary growth by metastasis or permeation through these channels. I was unable to exclude the invasion of the ovarian lymph vessels by the ovarian carcinoma. Such an incident, which frequently happens, would not interfere with the implantation of cancer cells both on the serosa and on the mucosa of the tube with the subsequent invasion of the underlying lymph vessels by the secondary carcinoma of the implants.

Secondary carcinoma of the mucosa of the fimbriae and adjacent ampulla of the left tube was found with evidence that the tumor in these situations had spread in the mucosal lymph vessels. It was also present in the lymph vessels of the mesosalpinx. The growth in the left tube was so far advanced that it was impossible to determine either the site or the pathogenesis of the initial secondary carcinoma in this situation.

Since the tubal mucosal lymph plexus is much richer than the serosal plexus and just as accessible as the latter, one might antici-

only 3 of these fimbriae were examined microscopically. Carcinoma was found in the lymph vessels of the ampullar mucosa in 3 of these 7 tubes.

How may we account for the large number of instances in which the secondary tumors were situated in the distal portion of the tube either in the fimbrial mucosa only or in both the fimbrial and ampullar mucosa? The fimbriated end of the tube is the portion of the tube nearest the ovary and therefore it should be the portion of the tube most likely to be invaded in the continuous extension of the ovarian growth. Since an anastomosis apparently exists between the efferent lymph vessels of the ovary and those of the distal portion of the tube, including the ovarian fimbriae, these vessels offer channels for the spread of the ovarian carcinoma to this portion of the tube. We also must realize that the mucosa of the fimbriae is the portion of the tubal mucosa most exposed to cancer cells which have escaped from the ovarian tumor into the peritoneal cavity. If implantation carcinoma occurs on the tubal mucosa one would expect to find it most frequently in the fimbriae. Since the fimbrial and adjacent ampullar mucosa have a common rich lymph plexus consisting of true capillaries without valves, it is to be expected that if carcinoma reached the vessels of one portion of this plexus by any route it might easily spread to other portions of the same plexus. This might account for the frequent occurrence of carcinoma in the lymph vessels of both the fimbrial and ampullar mucosa of the same tube.

Carcinoma was not found in the lymph vessels of the fimbrial mucosa in 6 of the 7 tubes in which the growth in the tubal mucosa was restricted to that of the fimbriae. If these 6 patients had not been operated upon the carcinoma of the tubal mucosa, later, might have invaded the lymph vessels of the fimbrial mucosa and spread from there to the lymph vessels of the ampullar mucosa and thus presented the picture so often seen in these cases.

Carcinoma of the tubal mucosa secondary to carcinoma of the ovary may arise in the following ways:

1. By the continuous extension of the primary ovarian tumor invading the fimbriae and replacing portions of its mucosa (see Figs. 43 and 50). Carcinoma in the mucosal lymph vessels in some of these cases may come primarily from the portion of the ovarian growth that has replaced the fimbrial mucosa. An extensive ovar-

Both tubes and ovaries and the uterus, the appendix and two epiploical appendages were removed. The patient recovered from the operation, had deep X-ray treatments, was temporarily improved, but eventually died of carcinoma. The peritoneal implants were carefully studied. The results of some of these studies have been presented in a previous paper⁹ (see Case 5 of that paper). Grossly the fimbriae of the left tube appear swollen and retracted (see Fig. 157). Implants are present on a mucosal fold of the fimbriae about a carcinoma which apparently started in or on the mucosal folds of the fimbriae around the abdominal ostium of the tube and had invaded the folds of the fimbriae and adjacent ampulla including their lymphatics (see Figs. 161, 162 and 163). For evidence that this carcinoma primarily may have been of implantation origin with subsequent invasion of the lymph vessels, see Figures 158 to 163 inclusive. Carcinoma was not found in the mucosa of the opposite tube.

THE PATHOGENESIS OF CARCINOMA OF THE TUBAL MUCOSA SECONDARY TO CARCINOMA OF THE OVARY

Carcinoma of the tubal mucosa secondary to carcinoma of the ovary was encountered in 23 patients. Since carcinoma was found in both tubes in 9 cases and in 1 tube only in the others, 32 tubes with secondary carcinoma of the mucosa, multiple in many instances, were studied. Carcinoma was detected in the fimbrial mucosa in 25 of these 32 tubes, only in the fimbrial mucosa in 7, and in the mucosa of both the fimbriae and the ampulla in 18. In 7 tubes the fimbriae were not involved but carcinoma was present in the mucosa of the ampulla.

The 7 tubes in which the carcinoma of the mucosa was restricted to that of the fimbriae were carefully studied. Carcinoma was found in the lymphatics of the fimbrial mucosa of only 1 of these tubes and, in this instance, well may have come from the spread of the secondary growth in the fimbrial mucosa and not from the ovarian tumor through the lymph vessels.

In the 18 tubes in which carcinoma was situated in the mucosa of both the fimbriae and ampulla, carcinoma was found in mucosal lymph vessels of both the fimbriae and the ampulla in 16.

In the 7 tubes in which carcinoma was found only in the ampullar mucosa, the fimbriae grossly appeared normal in all, but

growth through its lymph vessels as a sole or principal cause of the mucosal carcinoma was found in 6 tubes. In some of the cases in Group "1" as well as those in Group "5" lymphatic metastasis and permeation from the ovarian tumor may have played an important rôle.

3. In 2 tubes the lymphatic origin of the mucosal tumors apparently arose from the invasion of the lymph vessels of the mesosalpinx by carcinoma; in one instance from an extensive ovarian growth which had invaded the mesosalpinx, the isthmus of the tube and the uterine cornu, and in the other case from carcinoma implanted on the mesosalpinx.

4. Implantation of cancer cells on the mucosa was encountered in 16 tubes and was judged to be the sole cause of the secondary mucosal tumors in 9.

5. In 8 tubes the pathogenesis of the predominating mucosal tumor could not be definitely determined. Carcinoma was present in the lymphatics of 6 of these 8 tubes.

SUMMARY OF IMPLANTATION CARCINOMA OF THE TUBAL MUCOSA

Implants on the tubal mucosa were found in 12 of the 23 patients with carcinoma of the tubal mucosa secondary to carcinoma of an ovary and, in four instances, occurred in both tubes. They were multiple in 14 of the 16 tubes: frequently various stages in their development could be seen in a single tube. Implants were present on the fimbrial mucosa in 12 of the 16 tubes, in the fimbriae only in 7 and in both the fimbriae and ampulla in 5. In four instances they occurred only in the ampulla. None were found in the isthmus of the tube or on the uterine mucosa.

In 9 tubes implantation was judged to be the sole cause of the secondary carcinoma of the tubal mucosa and also well may have been the cause of the original secondary growth in 3 tubes in which the pathogenesis of this growth could not be positively determined. In 4 instances implants were associated with a secondary carcinoma of the tubal mucosa which obviously had reached the mucosa from the invasion of the fimbriae by an advanced ovarian growth or by lymphatic permeation. A secondary carcinoma of the tubal mucosa due to either of the above phenomena does not prevent the implantation of cancer cells on the mucosa of that tube.

The large number of tubes in which implants were found in the

ian growth may in like manner invade any portion of the tube (see Figs. 19 and 20).

2. By lymphatic metastases or permeation through lymph vessels coming from an ovary in which the primary growth has invaded the lymph vessels of that organ (see Figs. 13 to 17 inclusive).

3. By lymphatic metastasis or permeation arising from the lymph vessels of the mesosalpinx or wall of the tube which has been invaded by an ovarian carcinoma from without.

4. Through the veins (I have been unable to demonstrate this route).

5. By the implantation of cancer cells on the mucosa of the fimbriae and ampulla.

In some instances the determination of the pathogenesis of a given carcinoma of the tubal mucosa is easily made. At other times, especially when the growth is advanced, the diagnosis of its pathogenesis may be very difficult or impossible.

If the secondary tumor presents the histological picture of having arisen from an extensive ovarian growth invading the tubal mucosa, or from the implantation of cancer cells on the tubal mucosa, and if, at the same time, carcinoma is present in the mucosal lymph vessels, it may be impossible to determine whether the carcinoma in these vessels came directly from the tumor in the ovary by metastasis or permeation through the ovarian lymph vessels, or from a downstream spread of the secondary growth in the tube. It might come from both sources in a given case.

Frequently multiple tumors are present in the same tube. It is evident in some instances that the pathogenesis of all of the tumors in a given tube is not the same (see Figs. 43, 46, 47, 90 and 93).

An attempt has been made to group the 32 tubes with carcinoma of their mucosa secondary to carcinoma of the ovary according to the judged pathogenesis of the mucosal carcinoma or to that of the primary or predominating growth when multiple tumors were present.

1. A continuous extension of the ovarian tumor to the fimbriae of the tube replacing a portion of their mucosa was found in seven instances. Carcinoma was observed in the mucosal lymph vessels in all but one of these.

2. Evidence of metastasis or permeation from the ovarian

a local reaction in the subepithelial tissue and a loss of the overlying epithelium. In some instances fibrin is present, holding the cancer cells in place, and at other times fibrin is not evident (see Figs. 37, 111, 114 and 149). Cancer cells are also found attached to or becoming embedded in the outgrowths of granulation tissue just described (see Figs. 28, 30, 65 and 93). In these ways carcinomatous implants arise on the tubal mucosa just as they arise on the peritoneal serosa. As in the latter situation, the mature mucosal implants may be of the following three types: carcinoma replacing portions of the tubal epithelium as though grafted in it (see Figs. 32, 33, 34, 35, 58, 110, 114 and 150), and carcinoma either growing on the surface of or embedded in polypoid newly formed tissue which has arisen on the surface of the tubal mucosa (see Figs. 31, 29, 46, 47, 54, 66 and 70). All stages in the pathogenesis and growth of each of these types of implantation metastases are illustrated and described in this paper. I have not been able to ascertain the exact cause of the local reactions of the peritoneal serosa and of the tubal mucosa which form a fertile soil for the implantation and growth of cancer cells. It is obvious that cancer cells, in some way, are responsible.

Cancer cells in some of the mucosal implants die (see Fig. 47). In other situations, especially in the encapsulated polypoid implants, the carcinoma apparently grows slowly and may be confined to the implant for a long time (see Figs. 66 and 70). It has been shown that the carcinoma in serosal implants may invade the organ or structures beneath them, including the lymph vessels, just as a primary carcinoma invades and spreads. Why should not the carcinoma in mucosal implants do the same?

It has been shown that the granulation tissue on the serosa of one patient with peritoneal carcinomatosis contained lymph vessels, and that the carcinoma in the preexisting lymph vessels of the mesosalpinx from which this granulation tissue had arisen had spread into the newly formed lymph vessels of the granulation tissue (see Figs. 82, 83 and 84). This phenomenon was also observed in granulation tissue which had arisen on the fimbrial mucosa of this patient (see Figs. 94, 95 and 96).

It has been shown that newly formed lymph vessels are present in the stroma of some serosal implants. Circumstantial evidence indicates that carcinoma in these implants may spread through

fimbriae can easily be understood since the fimbrial mucosa is the portion of the tubal mucosa most exposed to cancer cells escaping from the ovarian growth into the peritoneal cavity.

Since cancer cells are frequently found in the lumens of the tubes of patients with carcinoma of the ovary, this portion of their mucosa also may be exposed to these cells. I have observed that they may reach the lumen of the tube in the following ways:

1. From the peritoneal cavity of patients with peritoneal carcinomatosis, through a patent abdominal ostium of the tube (see Figs. 108, 109, 137, 140, 141 and 143).

2. From an ovarian carcinoma in a tubo-ovarian cyst through the patent tubal ostium (see Fig. 23).

3. From the portion of an ovarian carcinoma which has invaded the fimbriae of the tube and then grown through the lumen of the tubal ostium into the lumen of the ampulla (see Figs. 50, 51 and 52).

4. From the invasion of the wall of the tube by an ovarian carcinoma and the subsequent extension of the growth to and through the tubal mucosa (see Fig. 20).

5. From metastatic carcinoma of the tubal mucosa of either lymphatic or implantation origin (see Figs. 92, 26 and 41).

Cancer cells escaping into the lumen of the tube by any route seem to be alive. They apparently may not only live a long time in this situation, but also may increase in numbers, as shown by the presence of mitotic figures in some instances, just as they live and multiply in the lumens of lymph vessels and in the peritoneal cavity. It is to be expected that these cells may become implanted on the tubal mucosa when a suitable soil is created in that mucosa for this phenomenon.

In the present study local reactions were found in both the fimbrial and ampullar mucosa similar to those seen in the serosa of patients with peritoneal carcinomatosis. The form and degree of these reactions varies in individual instances. Sometimes it is slight and consists of a local proliferation of the cells of the sub-epithelial tissues of the mucosa associated with a partial or a complete loss of the overlying epithelium. In other instances granulation tissue is present on the surface of the mucosa, usually in the form of sessile or pedunculated polypoid outgrowths. Cancer cells are found attached to the areas of the mucosa which show

these newly formed lymph vessels into the preexisting lymph vessels beneath the implant. It is shown in this paper that newly formed lymph vessels are also present in the pedicles of some mucosal implants (see Fig. 71).

One striking anatomical feature of the fimbrial and ampullar mucosa is the richness of its lymphatic plexus, apparently much richer than the lymphatic plexus of the serosa of the ampulla of the tube. It is apparently more superficial and, therefore, more accessible to injury and stimulation than the latter. When one encounters a secondary carcinoma of the tubal mucosa with the histological structure of an implant and carcinoma in the lymph vessels of the mucosa beneath this tumor with evidence that the carcinoma in the lymph vessels is either continuous with or closely related to the secondary mucosal carcinoma, one is tempted to believe that this secondary carcinoma is an implant and that cancer cells from it may have spread into the underlying lymph vessels (see Figs. 107, 111 and 138). The cancer cells from an implant might, in this way, spread to the lymph vessels of the mesosalpinx and even reach the ovary, the site of the primary carcinoma from which the original cancer cells of the implant came.

Since carcinoma in the preexisting lymph vessels of the mesosalpinx and tube sometimes spreads into the lumens of newly formed lymph vessels in granulation tissue arising on both the serosa and the tubal mucosa (see Figs. 82, 83, 84, 94, 95 and 96), it might subsequently invade the granulation tissue about these vessels. A condition would thus arise simulating the extension of the carcinoma in an implant into the newly formed lymph vessels of its stroma. I have never recognized this phenomenon in granulation tissue on the tubal mucosa, but have seen it in similar tissue on the serosa. A still more confusing picture would arise if cancer cells became embedded in the granulation tissue just described before or after the spread of carcinoma in preexisting lymph vessels into the newly formed lymph vessels in the granulation tissue.

A differential diagnosis between mucosal implantation metastases and metastases to the tubal mucosa through the lymph vessels usually may be made easily in the early stages of these secondary tumors. However, in the advanced stages of either of these metastases a positive diagnosis of their pathogenesis may be very difficult or impossible.

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ing; and (2) in dementia paralytica. (*F*) *Diminution of vascular caliber* is found in simple atrophy ("abiotrophy") of nervous structures.

Discussion

(Dr. Joseph Tannenberg, Albany.) During the last year I made direct observations on the brain during anoxic shock when the surface of the brain was exposed by trepanation. The wound in the skull was covered by a celluloid plate. The gas mixture containing a decreasing percentage of oxygen was administered by means of a gas mask. When the anoxemia was extended so far that the respiration became gasping, a relatively sudden swelling of the brain occurred so that the brain was pushed into the wound of the skull tightly against the celluloid plate. I had the impression that this was due to a definite edema of the brain, but was greatly surprised that the brain shrank again to its former volume within about a half minute when the anoxemia was interrupted. This was also noted when the same experiment was repeated five times, one after another, and even when the rabbits were finally killed by pure nitrogen. The extreme swelling continued only until death. Then simultaneously with the last constriction of the arteries the brain shrank again so much that its volume seemed to be smaller than at the beginning of the experiment. From these observations I drew the conclusion that it is almost impossible to gain a real opinion of the reactions of the brain arteries during life from a study of a fixed brain, even when the best histological methods are employed.

(Dr. N. Chandler Foot, New York City.) I think it might be well if Dr. Alexander would give us a few pointers on the technic of this method. We would like to know about it.

(Dr. Alexander.) In reply to Dr. Tannenberg, I wish to state that we were familiar with his excellent work on vital observations. It is a splendid piece of work on the subject. Vital observations are superior in many respects to what we can see postmortem, but there are distinct limitations to it also. A direct observation of these phenomena in the living human brain is only possible for the neurosurgeons, but they, like the experimental physiologists, are limited to observations of vascular phenomena at the surface of the brain, because the intracerebral vessels cannot be visualized satisfactorily in the living except with the Knisely technic, which is not applicable to larger mammalia or to man. In our own animal experiments we ourselves and Dr. A. C. P. Campbell have found the circulatory disturbances caused by local anaphylaxis, electric shock, asphyxia, and various anesthetics faithfully preserved in our benzidine stained preparations. The more material we examined, the more we became convinced that agonal and postmortem phenomena do not significantly alter the picture. It may be that there is some loss of blood from the arteries due to terminal constriction, but this constriction is usually not forceful enough to move the blood column in the precapillary arterioles and in the capillaries, or in the veins of the brain. The state of filling of the smaller vessels, however, is the most important in the interpretation of a benzidine picture. We have convinced ourselves that the benzidine stain, if performed and interpreted with the necessary precautions, reveals true ante mortem pathological phenomena. The beaded vascular channels of a brain abscess or of a focus of softening, the varicose deformities in alcoholic polioencephalitis, or the sinusoids of brain tumors are of course not significantly modified by agonal or postmortem phenomena.

As to Dr. Foot's question, the stain is essentially the benzidine reaction, such

as is used for demonstrating blood in the stool. There are a few additional steps necessary for its application to tissue sections. I shall be glad to send an outline of our modification of the stain to anyone interested.

INFLAMMATION OF THE PETROUS PYRAMID OF THE TEMPORAL BONE. Andrew A. Eggston, New York City.

Abstract. The paper will consider the technical preparation of serial sections of the labyrinth and petrous bone. There will be a review of the pathways of infection to the petrous pyramid through the regional venous circulation and through the chain of pneumatic cells connecting with the mastoid and tympanic cavity.

A discussion of the types of inflammation usually found, namely an osteomyelitis or osteitis, depending on which structures are chiefly involved, is presented.

Abscess formation in this region with subsequent rupture or invasion of the dura and other intracranial structures, resulting in meningitis or brain abscess, is demonstrated. Several cases with lantern slides illustrating various types of pathological conditions in the labyrinth or petrous bone are presented.

Discussion

(Dr. Howard T. Karsner, Cleveland.) Will Dr. Eggston explain the relation, if any, between pneumatization of the petrous portion of the temporal bone and extension of suppuration from the mastoid cells to the pyramid and its apex?

(Dr. Eggston.) About 33 per cent of mastoids are pneumatized, but pneumatization is not necessary for the infection to spread to the petrous pyramid. As I tried to show in the slides, the infection can spread by means of the veins or lymphatics, or even by contiguity of tissues from the tympanic cleft into diploic bone and result in an abscess. Therefore it is not absolutely necessary for the petrous pyramid to be pneumatized in order to become involved in an inflammatory process.

DEGENERATIVE ARTHRITIS. A COMPARISON OF THE PATHOLOGICAL CHANGES IN MAN AND EQUINES. George R. Callender and R. A. Kelser, Ancon, Canal Zone.

Abstract. The changes of degenerative arthritis found in 60 knee joints from man and in various joints from 54 horses and mules are presented.

The lesions were of practically identical types in man and equines though advanced degeneration was seen only in man, as the equines develop such disability that they are destroyed before such advanced changes occur.

The primary change was found to be in the peripheral portion of the cartilage and consisted of swellings, grooves, blisters and fibrillation of the matrix. These were followed by necrosis, loss of substance and ulcer formation.

Hypertrophic changes were considered to be secondary to loss of substance and consequent malocclusion of the joint and are believed to be of a compensatory effort to maintain the proper distribution of the joint stress. This hypertrophy consisted of bone growth replacing destroyed cartilage and extending joint surfaces. It appeared to start in the calcified matrix and to extend into overlying degenerated cartilage.

Considerable joint change was often found in both man and animals without there being any history of symptoms.

The incidence of degenerative arthritis in this small series in man agreed with that of Keefer and Parker. None was found in persons below 20 years of age; there was rapid increase up to the age of 40, and thereafter some change was found in practically every knee joint examined.

Only 1 horse below the age of 10 years was examined and that one was the only one without joint lesions.

Discussion

(Dr. Herbert Fox, Philadelphia.) This splendid paper by Dr. Callender and Dr. Kelser is another example of the use of comparative pathology. It happens to hinge with a subject that I have been studying, the first part of which is just completed. Having looked over some 1400 skeletons and 250 autopsies on the lower mammals for evidences of organic and systemic arthritis, it was discovered that the variety to which the horse belongs has the least arthritis. The split-toed ungulates have distinctly more arthritis than the solid-toe ungulates. The large animals on stilt-like legs have more arthritis in the posterior extremities. In other quadrupeds and those that stand up sometimes, like the monkeys and the apes, there is arthritis in the front legs. The jolt-shock reaction appears to have something to do with the localization of arthritis. This disease appears more after maturity, which is when reversibility of tissue reaction disappears and irreversibility becomes prominent. Both osteoarthritis and rheumatoid arthritis were found.

The lesions in the ungulate group were comparable to those described by Dr. Kelser. Carnivorous animals show the greater destruction of the articular surfaces. In the ungulate group the periarticular tissues are more involved than the true articular tissues, and from a small number of histological sections would seem to agree with the work reported by Dr. Kelser, especially in that the process seems to be inflammatory from within the bone toward the direction of the articular cortex.

The terminology "degenerative arthritis" begins with Richardson *et al.* in 1912, and the term "atrophic arthritis" was adopted shortly later. The English use "osteoarthritis" and "rheumatoid arthritis"; I believe this is better. The group of students represented by Hench and Bauer adhere to the American usage of "atrophic" and "degenerative."

Dr. Kelser's slides seem to show that the lesions depend on inflammation coming from within the bone ends and proceeding toward the joint space. This helps to support the thought that what Dr. Kelser terms degenerative is end-osteal in origin. I happen to belong to the group that considers the two forms as differing expressions of rate and intensity rather than of specificity. Certainly the lesions from the carnivorous animals, particularly the felines, show very early changes below the articular surface as distinct as those in the periarticular tissue.

This is a very important paper and such comparative studies will give considerable help in explaining why we have arthritis and why it is localized.

(Dr. D. Murray Angevine, New York City.) Has Dr. Kelser any figures on the incidence of osteoarthritis or degenerative arthritis in the Negro, as compared to whites? Probably a good many of the autopsies were done on negro patients.

I should also like to know the findings in the synovia in this group of cases,

and finally, I should like to know what the incidence of infectious or rheumatoid arthritis in Panama might be.

(Dr. Kelsner.) A number of our cases in these 60 men were whites. Clark did a preliminary survey on a large number of blacks some years ago. He believes that the high incidence of degenerative arthritis in negroes is due to weight-carrying, as has already been indicated. Our study included a number of white soldiers who came to autopsy.

We made cultures from all the equine cases. There was no evidence of any bacteria. The synovial fluid and scrapings were cultured aerobically and anaerobically. The fluid was clear and we had no evidence to indicate that there was any infection.

The incidence in Panama, as compared to that in other places, I believe is probably no higher. The point is, in Panama the pathologist at the Gorgas Hospital had not routinely opened joints, and just 5 months before he left we got him to open joints when it was feasible, and found this high rate, but I believe if joints were opened routinely in a large number of hospitals in connection with autopsies that we would find a much higher rate than is suspected. We hope to be able to continue this study from the comparative standpoint in Panama and some of the other Army hospitals in the States to see if we can get a line on the rate.

OSSEOUS CHANGES IN MAN FOLLOWING THE PROLONGED INGESTION OF FLUORIDES. John T. Bauer, Philadelphia, Pa.

Abstract. The apparent increase in the density of the bones of cryolite workers was noted by roentgen-ray in 1932 by Møller and Gudjonsson (*Acta radiol.*, 1932, 13, 269) who ascribed this change to the prolonged ingestion of fluorine, a constituent of the mineral. Similar changes were noted and reported by Bishop (*Am. J. Roentgenol.*, 1936, 35, 577) in a negro 48 years old who had worked for 18 years in a fertilizer factory handling finely ground rock phosphate, a sample of which contained 3.88 per cent fluorine. These changes radiographically were as follows: (a) increase in opacity of the bones without alteration of the normal bone structure; (b) lack of normal sharpness of the bone outlines; and (c) extension of the calcification into the ligamentous attachments. The changes were greatest in the bodies of the vertebrae and least in the long bones. The patient's death from syphilitic aortitis permitted a pathological and chemical examination of the bones. Pathologically the cortical portions of the bones were thicker than usual and encroached on the spongy marrow spaces. Osteophytes, some of which bridged the cartilaginous discs of the bodies of the vertebrae and became fused, were present in many bones, especially along the tendinous insertions of the muscle. The interosseous ridges were thickened and roughened. Chalky white patches of bone were seen over the sternum and elsewhere. Microscopically, there was an increase in the amount of bone that compressed the haversian canals. No granular deposits were seen (Bauer, Bishop and Wolff: *Bull. Ayer Clin. Lab., Pennsylvania Hospital*, 1937, 3, 67). Chemical studies of the bones by Wolff and Kerr (*Am. J. M. Sc.*, in press) revealed normal values for ash, calcium, phosphorus and carbon dioxide. That of fluorine was from 10 to 20 times normal. Despite the increased radio-opacity, the specific gravity of some of the fluoride bones was less than that of normal bones, hence the former was probably due to an increase in the amount of bone present.

From the recent review of Roholm (Fluorine Intoxication, 1937, H. K. Lewis

& Co., Ltd., London) it is evident that pathological studies of chronic fluorine poisoning other than dental fluorosis in man have been few, and the present one is the first to be reported from this continent.

Discussion

(Dr. John W. Williams, Cambridge, Mass.) I should like to ask what the blood findings were.

(Dr. Bauer.) The blood findings in this patient were normal, and that has been true of practically every one of the patients observed. The greatest amount of work has been done by the Danish workers, and Roholm's review which has recently appeared refers to practically all of the studies. Anemia was not common in these studies.

THE CARDIORENAL TOXIC PROPERTIES OF THE TOXIN OF VIBRION SEPTIQUE IN ANIMALS. J. G. Pasternack, New Orleans, La.

Abstract. The anaerobe vibron septique produces a specific toxin which is unusually rapid and dramatic in its action when injected intravenously into laboratory animals. The quick injection of 0.03 cc. of potent toxin may be fatal to a rabbit in 30 seconds. A period of incubation is almost non-existent, or so short that the question has been raised whether the action is that of a true toxin. Its mode of action has also caused some speculation.

In this report results are presented of investigations of the pathological physiology and pathology produced by the toxin of vibron septique in rabbits, guinea pigs, pigeons and mice injected subcutaneously, intramuscularly and intravenously with various doses of toxin.

Of interest was the determination of the relation of the effects produced to the survival time and to the size of the dose of toxin used. Symptoms may develop in 1 to 3 minutes and death may occur in 4 to 6, depending on the potency of the toxin used. It has been stated that the toxic dose for rabbits followed the "all" or "none" law, *i.e.* the smallest amount of toxin which was fatal reacted as quickly and potently as a considerably larger fatal dose. In the present study the survival time was found to be roughly inversely proportional to the dose of toxin. There were exceptions, and often variations occurred with the same quantity of toxin in animals of the same species. It was found that the incubation period could be considerably increased by closely approaching the M.L.D. for the toxin used. If the toxin is a potent one and the doses are far apart, the M.L.D. may be missed and the result is either prompt death of the animal or survival without symptoms.

The peculiarity of variation in the length of survival of animals that received the same quantity of toxin is difficult to explain. The possibility was considered that the rapidity with which the toxin was injected might be a factor. Within the limits of the experiments the survival time had no relation to the rapidity with which the injection was made.

Carotid blood pressure readings, kymographic studies and perfusion experiments indicated that the death of the animal was caused by the action of the toxin on the heart, and that respiratory symptoms were of secondary nature. Detailed gross and microscopic studies of the pathological anatomy produced in animals dying at various intervals after various doses of toxin verified the cardio-toxic properties of the toxin of vibron septique.

Most of the animals that survived as long as 6 minutes showed some type of

degenerative lesion in the heart. In intoxications of short duration only small, scattered degenerative changes were demonstrable. In animals surviving a number of hours or several days, extensive areas of the myocardium, or rarely the whole heart, showed advanced Zenker's "waxy degeneration." Conduction tissue was demonstrated in 3 animals, but only 1 showed degenerative lesions.

The toxin of vibrión septique also has severe nephrotoxic properties. The severity of the lesions produced varies more or less with the duration of the intoxication; the longer the animals survived, the more marked were the lesions. The lesions were degenerative, thrombonecrotic and hemorrhagic in character.

Severe acute lesions occurred also in other organs, but less regularly than in the heart and kidneys.

Since all studies indicated that the principal action of the toxin of vibrión septique was on the heart, an experiment was designed to determine whether the toxin has a special affinity for cardiac muscle. The results showed that *in vitro* very little toxin actually combined with the muscle tissue.

Subcutaneous and intramuscular injections of the toxin, in doses many times greater than the M.L.D., by the intravenous route, usually resulted only in local necrosis, repair and scar formation, and but rarely in death.

Discussion

(Dr. Norbert Enzer, Milwaukee.) Has Dr. Pasternack any explanation for the staining of the blood vessels by hemolyzed blood which is such a common finding in these autopsies?

(Dr. Theodore J. Curphey, New York City.) Have similar studies been made with diphtheria toxin?

(Dr. Wiley Davis Forbus, Durham.) I should like to know if this is not a lesion comparable to that produced by the hemolytic staphylococcus toxin so much talked about of late, and described rather carefully by Rigdon and Joyner.

(Dr. Pasternack.) In answer to Dr. Forbus the kidney lesion is closely comparable with that of the hemolytic staphylococcus toxin.

With diphtheria toxin similar lesions in the heart may occur, but not so quickly or so extensively.

The toxin of vibrión septique has marked hemotoxic properties and considerable free blood pigment is liberated.

THE RENAL LESION IN CYTOTOXIC GLOMERULAR NEPHRITIS (IN RABBITS). William E. Ehrich, Philadelphia, Pa.

Abstract. The nephritis was produced by the method of Masugi. Ducks were immunized with an emulsion of the kidneys of rabbits, and the antikidney serum thus obtained was injected intravenously into rabbits. The essential lesion was a diffuse cellular proliferation within all the glomeruli in all animals studied. In some animals this lesion was complicated by the deposition of fibrin in the glomerular spaces and later, as a result thereof, by adhesion or crescents. Since the deposition of fibrin and the formation of crescents were found as early as the proliferation, they can no longer be looked upon as an essential characteristic of a certain phase of glomerular nephritis. Other changes were erythrocytes in capsular spaces and tubules, hyaline casts, hyaline droplets in convoluted tubules, and, in a few cases, fatty degeneration. Clinical changes were edema, albuminuria, oliguria, diminished filtration, rise in blood urea nitrogen, and so on. Since the author is unaware of any discrepancies between the experimental

disease and human nephritis, it is believed that the two are identical. During the first week of the experiment there was an excellent diuresis and an increase in filtration, while the glomeruli were rather hyperemic. Oliguria, decrease in filtration and ischemia of the glomeruli became manifest only after 8 days, when the anatomical changes were fully developed. It is believed, therefore, that glomerular nephritis starts with a latency period, and that it does not start with a spastic condition in the kidneys, as maintained by Volhard.

Discussion

(Dr. J. E. Smadel, New York City.) Acute nephrotoxic nephritis in the rat presents a picture somewhat different from that shown by this experimental disease in the rabbit. The occurrence of fibrin thrombi in the glomeruli and erythrocytes in the urine do not depend, in the rat, upon the effect of nephrotoxin (the organ-specific antibody); instead these findings result from non-organ-specific factors. Another difference to be noted between the response of these two species to this type of renal injury lies in the amount of endothelial proliferation present in glomerular tufts. Such proliferation occurs rarely, if at all, in rats that have a true nephrotoxic nephritis; on the contrary, the typical glomerular lesion in this case is swelling of the glomerular capillary basement membranes. Still another point of difference has to do with the time of onset of nephritis after injection of antikidney serum. Manifestations of the experimental disease are evident in rats within a few hours while a latent period of a week intervenes in rabbits.

Dr. Ehrich has not emphasized the progressive nature of this nephritis in rabbits. Rats of a certain strain almost invariably pass over from the acute experimental disease into a chronic nephritis. The majority of these animals show clinical, functional and histological evidence of progressive renal involvement. Dr. W. M. Arnott, when he was in this country recently, said that he did not believe that rabbits develop a truly progressive kidney lesion following the injection of antikidney serum. I should like to ask Dr. Ehrich's opinion on this point.

(Dr. E. T. Bell, Minneapolis.) I am interested in work of this type. It was not clear to me from the presentation whether this was a diffuse or focal glomerulonephritis. Apparently in the slides shown there was a good deal of difference in the intensity in different parts of the field. It is clear you can produce glomerular lesions in a variety of ways. I do not think we should conclude from this that allergy is the mechanism of human glomerulonephritis. Inasmuch as we see acute glomerulonephritis developing so frequently within 2 or 3 days after an initial infection, it is hard to believe that sensitization is necessary for the development of the disease. We must be careful to distinguish focal lesions with uneven distribution from a true diffuse nephritis.

(Dr. Ehrich.) As to the first question, rats and rabbits are different in many respects. As to the outcome of the disease in our milder cases, we had complete recovery. We have not studied the chronic ones to the stage of death. We have animals on the way, and I believe that at least those cases that had a great deal of fibrosis — 80 to 90 per cent of the glomeruli were completely fibrosed — may eventually die from uremia.

As to Dr. Bell's question, we are dealing with a diffuse proliferation where all the glomeruli are affected. There is no discrepancy between the disease experimentally produced in rabbits and human glomerulonephritis.

As to the etiology of the disease, we have drawn no conclusions.

ACUTE HEMATOGENOUS INTERSTITIAL NEPHRITIS. Paul Kimmelstiel, Richmond, Va.

Abstract. Six cases of acute hematogenous interstitial nephritis are presented, all of which show more or less marked isosthenuric oliguria or anuria and rise of the non-protein nitrogen in the blood. Twelve other cases of interstitial nephritis which were found incidentally at autopsy are included.

The morphological characteristics of this lesion are described. It is emphasized that even in the early stage the interstitial exudate contains plasma cells, lymphocytes, eosinophils and monocytes, in addition to polymorphonuclear cells. A distinction between focal and diffuse interstitial nephritis cannot be made.

The conditions causing interstitial nephritis are listed. In addition to the infectious diseases and septicemia, it is found to follow conditions associated with hemolysis, in particular blood transfusion with incompatible blood. It is also found in cases of hepatorenal syndrome with infectious and non-infected liver injuries.

The correlation of functional disturbances with interstitial nephritis is discussed. It is concluded that the anuria does not result from renal edema by pressure on the tubular apparatus, nor is it caused by blockage of tubules if associated with hemoglobinuria. General or local renal circulatory disturbances are held the most likely cause of both oliguria and lack of concentration power.

Hematogenous interstitial nephritis is regarded as an allergic hyperergic reaction to foreign proteins or protein split products coordinated rather than causatively connected with hyposthenuric oliguria and anuric uremia.

Discussion

(Dr. E. T. Bell, Minneapolis.) I think we can explain the low specific gravity of the urine in this type of renal disease on the decrease in the number of functioning units left in the kidney. That is the explanation of low specific gravity in chronic glomerulonephritis. In this disease a large proportion of the glomeruli and tubules are blocked off by the infiltration. We get exactly the same inability to concentrate experimentally if we take out one kidney and about one-third of the other. The remaining kidney is perfectly normal, still the animal is unable to form a concentrated urine, and I think that is the basis here. If the anuria were due to heart failure, the urine would be of normal concentration.

(Dr. Howard T. Karsner, Cleveland.) It is of interest that there is little apparent tubular disease in association with the hyposthenuria in these patients and I think the explanation offered by Dr. Bell is probably sound. Dr. W. T. Councilman, who described the disease many years ago, attributed causative significance to alcoholism and exposure to cold. What is the relation of those factors to Dr. Kimmelstiel's cases? Dr. Councilman referred to the presence among the infiltrating cells of a type which he referred to as myeloid, not necessarily originating in the bone marrow. Were they present in these cases? I have recently seen a specimen in which both mononuclear and polymorphonuclear eosinophiles were present and am interested to know Dr. Kimmelstiel's observation of these cells in his cases. Did he find any instance in which the infiltration was diffuse rather than focal?

(Dr. Virgil H. Moon, Philadelphia.) I feel that the cases of anuria which were presented may fall into more than one group, so far as etiology is concerned. I am interested particularly in the last case, the individual who suffered

a severe burn and who died in shock. It has been our experience both in experimental and in clinical shock that there is marked hemoconcentration accompanied by oliguria or anuria. Several authors have cited the observation that the glomeruli of the kidneys are unable to secrete urine from a markedly concentrated blood. Perhaps also the blood pressure, which is low in shock, is a contributory factor. I do not believe all cases of acute anuria will be shown to be of one cause, and certainly a number of them are incident to the shock syndrome and are probably explainable on the basis just suggested.

(Dr. Irving Graef, New York City.) Will Dr. Kimmelstiel indicate whether or not similar interstitial changes were found in the other organs, especially with reference to those cases which were complicated by infection?

(Dr. H. Edward MacMahon, Boston.) I should like to ask Dr. Kimmelstiel if there was any striking increase in size of the kidneys that he has reported, and, as he has said nothing of the gross appearance of these kidneys, I wanted to ask if there was any marked change in consistence or if the capsules appeared unusually stretched. I have recently seen the kidneys from a patient who died shortly after a transfusion. Preceding death there had been a period of oliguria followed by complete anuria. Each of the kidneys weighed 235 gm. Each was greatly enlarged but retained its natural contour. Each was unusually tense, and on incising the capsules they stripped spontaneously, indicating a good deal of tension of the kidney itself. Histologically the most striking change was an acute inflammatory edema of the stroma (acute interstitial nephritis). In the pyramids, and especially near the tips, the collecting tubules were completely compressed by this tension in the surrounding soft tissues. There were comparatively few inflammatory cells within the stroma. The tubules did show regressive changes and hemoglobin was demonstrable in some of their lumens. Such a finding at autopsy does suggest that surgical decapsulation might possibly have been of some benefit.

(Dr. Kimmelstiel.) I do not think that I can answer the questions on the pathogenesis of interstitial nephritis and the mechanism of anuria.

To come back to Dr. MacMahon's question first, I do not believe that increased intracapsular pressure causes anuria by compression of tubules. Although many of the cases showed marked increase of weight and the parenchyma was very moist, not all of them showed gross evidence of edema. Secondly, the tubules may not be distended nor are the glomerular spaces always widened.

As to Dr. Moon's suggestion that some of the cases may perhaps be looked upon as manifestation of shock, I really thought at first that they all could be explained in this way, particularly because I thought that this reaction might possibly be — if I venture to say so — an allergic hyperergic reaction to protein-split products which play a part in shock. Not all the patients, however, died in shock. Some of them showed hemoconcentration; others did not. Some showed falling blood pressure, others were normal. Some showed hypertension. Although Fahr and others think that interstitial nephritis with anuric uremia is a disease entity, I have come to the conclusion that this is not true.

As to Dr. Karsner's remarks, I admit that I did not know of Dr. Councilman's paper on the subject, but Lindau stated the same: he says that the foci of interstitial infiltration look like hematopoietic foci, and also used the expression "myeloid cells."

Personally I have had no experience with exposure and alcoholism.

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READ BY TITLE

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- HOW TO DIAGNOSE CANCER EARLY. William Carpenter MacCarty, Rochester, Minn.
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CHEMOTROPIC REACTION TO VARIOUS MICROORGANISMS OF POLYMORPHO-
NUCLEAR LEUKOCYTES OBTAINED FROM PATIENTS SUFFERING FROM ENDO-
CARDITIS LENTA AND CHRONIC OSTEOMYELITIS. William B. Wartman and
(by invitation) Edward A. Votypka, Cleveland, Ohio.

PARAGANGLIOMA OF THE THYROID. REPORT OF A CASE. F. W. Wigglesworth,
Montreal, Canada.



EARL BALDWIN MCKINLEY

1894-1938

EARL BALDWIN MCKINLEY, President of the American Association of Pathologists and Bacteriologists, was lost with the passengers and crew of the Hawaii Clipper, in the Pacific Ocean, July 29, 1938. He was born September 28, 1894. He obtained his A.B. in 1916 and his M.D. in 1924, from the University of Michigan. He was an officer in the front lines during the World War. Successively assistant in bacteriology and instructor in biochemistry at his alma mater, associate professor of pathology and professor of hygiene and bacteriology at Baylor University, National Research Council Fellow in Brussels, assistant and associate professor of bacteriology at Columbia University, New York, field director of the International Health Board in the Philippines, professor of bacteriology and director of the School of Tropical Medicine in Puerto Rico, he became professor of bacteriology and dean of the School of Medicine, The George Washington University, Washington, D. C., in 1931 and held that post at the time of his death. Early in his career he came under the influence of such men as Novy, Bordet, Jobling and Gay, to whom he repeatedly acknowledged indebtedness for inspiration and guidance.

McKinley was noteworthy as investigator, organizer and administrator. His research work covered a wide variety of subjects, but his especial interests were in the fields of filterable viruses and leprosy, in which he furnished highly significant contributions.

In Manila, he reorganized the health laboratory of the Bureau of Science. At Columbia, he established a course on filterable viruses. In Puerto Rico, he organized the department of bacteriology and sponsored the establishment of the Puerto Rico Journal of Public Health and Tropical Medicine. In Washington, he reorganized the School of Medicine, established the Theobald Smith Lectures and formed the Washington Academy of Medicine. He was foremost in the foundation of the Academy of Tropical Medicine.

He was a member and active in many scientific societies here and abroad. His work as a member of the Medical Advisory Board of the American Leprosy Foundation and as a member of the Executive Committee of the American Association for the Advancement of Science, will ever be gratefully remembered. His vision and advice were invaluable in the Council of the American Association of Pathologists and Bacteriologists.

The fact that he was only forty three years old when he was lost testifies to the inexhaustible energy of the man. He abounded in vitality, constructive imagination and initiative. Fatigue seemed unknown to him. He was generous of soul, as reflected in his kindly opinions of men and affairs. His capacity for friendship was unlimited. His genial personality, his cordial hospitality, his wide experience, his unfailing sympathy, his catholicity of taste and his deep interest in scientific matters made his home a salon for visitors from all over the world. He has left to science and medicine a treasure-house of contributions which will be a permanent memorial to the man and his works.

HOWARD T. KARSNER,

Secretary



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A CLINICAL AND PATHOLOGICAL STUDY OF SUBACUTE AND CHRONIC GLOMERULONEPHRITIS, INCLUDING LIPOID NEPHROSIS *

E. T. BELL, M.D.

(From the Department of Pathology, University of Minnesota, Minneapolis, Minn.)

In a previous report ¹ the various types of acute nephritis were described. This publication is based on a study of 181 cases which have been classified in accordance with certain clinical and pathological features as follows:

Group I. Subacute glomerulonephritis, 16 cases.

Group II. Chronic glomerulonephritis in which death was due to an intercurrent disease, 8 cases.

Group III. Advanced chronic glomerulonephritis of azotemic type, 117 cases.

A. With a history of acute glomerulonephritis, 30 cases.

B. No history of acute nephritis; kidneys weighing together 250 gm. or more, 33 cases.

C. No history of acute nephritis; kidneys weighing together less than 250 gm., 54 cases.

Group IV. Chronic glomerulonephritis of the hydropic type, 40 cases.

A. With the glomerular structure of chronic proliferative glomerulonephritis, 6 cases.

B. With a glomerular structure largely of membranous but partly of proliferative type, 9 cases.

C. With a normal glomerular structure or a membranous type of glomerulitis, 25 cases.

* Received for publication June 16, 1938.

The distribution of the cases by groups and by decades is shown in Table I. It is to be noted that this is the age at death and not the time of onset of the disease. When a complete history is available it is usually found that the disease has been present a number of years. In the 30 cases of the chronic azotemic type in which the complete course of the disease is known (Table IV) the average age of onset is 20 years and the average age at death 30 years. About two-thirds of the patients die between the ages of 20 and 50 years, and since most of them are under treatment only during the advanced stages, the grouping shown in Table I cor-

TABLE I
Frequency and Age Distribution of Nephritis

Age	Total number of autopsies	Groups				Total
		I	II	III	IV	
<i>yrs.</i>						
0-10	4,710	1	0	1	9	11
10-20	1,103	3	0	10	3	16
20-30	2,186	3	2	30	6	41
30-40	2,032	2	1	33	10	46
40-50	4,000	2	1	23	6	32
50-60	4,151	3	2	10	3	18
60-70	4,076	1	0	8	2	11
70-80	2,590	1	1	2	1	5
80-90	700	0	1	0	0	1
90-100	50	0	0	0	0	0
Total	25,598	16	8	117	40	181

responds fairly well with patients under observation. The disease is by no means limited to children and young adults, although it is probable that a majority of the cases have their onset during these periods. There are, however, instances in which the disease begins in middle life or later. In our experience about one-half of the cases of fatal nephritis in children under 10 years of age are the hydropic type (lipoid nephrosis).

Frequency: It appears in Table I that the forms of nephritis under discussion are relatively rare and comprise only about 0.7 per cent of the total mortality. Chronic Bright's disease, which includes hypertensive renal insufficiency as well as all forms of chronic glomerulonephritis, causes only a little over 1 per cent of all deaths. The high incidence of nephritis in vital statistics is

due to the inclusion of a large number of cases of primary hypertension with albuminuria and edema resulting from myocardial failure.

Sex: In the entire group studied there were 105 males and 76 females, but in the postmortem series there are approximately twice as many males as females over 30 years of age. When a correction is made for this factor there seems to be no predominance of either sex.

GROUP I. SUBACUTE GLOMERULONEPHRITIS (TABLE II).

This group blends on the one side with the acute and on the other with the chronic type, and the limitations are somewhat arbitrary. Clinically cases that terminate in uremia after a course of a few months duration are usually called subacute. Pathologically fairly definite diagnostic criteria have been established. The kidneys are not contracted; they are either of normal size or enlarged. Microscopically one finds a severe and uniform obstruction of all the glomeruli, but there are no hyaline glomeruli (Fig. 1). There is a moderate uniform atrophy of all the tubules. Subacute nephritis is distinguished from the acute form by the presence of moderate tubular atrophy; there is very little atrophy in the acute type. It is distinguished from chronic nephritis by the moderate uniform tubular atrophy and the absence of hyaline glomeruli; in the chronic form there are patches of extremely atrophic tubules associated with hyaline glomeruli (Fig. 5).

The 16 cases listed in Table II were identified by their pathological features but the clinical duration was known accurately in nearly all of them.

Example of Subacute Glomerulonephritis (Case 2, Table II)

Clinical History: A boy, 16 years of age, was admitted to the hospital Sept. 16, 1937. There was no history of scarlet fever. He had had occasional attacks of sore throat, but there had been no infection of any kind during the year preceding his present illness. In the latter part of June, 1937, he first noticed a slight swelling of the face and ankles, otherwise he was well at this time. About July 9th, he began to feel drowsy and weak; the swelling of his face and ankles had not increased. He consulted a physician who diagnosed nephritis. In August he spent 2 weeks in a hospital under the diagnosis of acute nephritis. About September 5th a generalized anasarca developed, with weakness, headache, vomiting and drowsiness. After September 11th he noted a disturbance of vision and oliguria.

TABLE II

Group I. Subacute Glomerulonephritis

Case No.	Autopsy No.	Age yrs.	Sex	Duration mos.	Blood pressure mm. Hg.	Albuminuria	Edema	Urea nitrogen mg./100 cc.	Phenolsulpho- nephthalein	Weight of heart gm.	Weight of kidneys gm.	Passive congestion of liver	Hemoglobin	Epithelial crescents	Comment
1	25-798	8	F	2	—	—	2	—	—	149	224	0	—	4	Casts. Plasma proteins 4.2 gm. %
2	37-1830	16	M	3	192/112	4	3	77.8 (1 day)	—	360	710	0	50	2	Retinitis
3	30-1567	16	M	7	180/114	4	3	116 (1 wk.)	0 (1 wk.)	320	285	0	45	1	Retinal detachment
4	31-1508	17	F	6	200/112	—	3	56.4 (1 mo.)	—	390	225	2	—	1	
5	18-118	21	M	7	140/80	+	2	14 (6 mos.)	15 (6 mos.)	375	425	1	—	3	
6	18-237	22	F	4	220/120 (1 wk.)	3	3	33 (12 days)	—	300	260	2	—	3	
7	13-8	26	F	3	—	+	1	—	—	Normal	Normal	0	—	4	Marked hematuria
8	12-131	33	M	3+	—	+	1	—	—	460	415	2	—	1	
9	16-368	39	M	5	—	3	3	104 (1 day)	0 (1 day)	375	280	0	35	2	
10	25-945	41	M	2	248/102	1	2	80 (7 wks.)	10 (7 wks.)	—	335	0	—	0	Embolic type
11	22-554	43	M	6	140/60 (1 day)	3	1	119 (1 day)	—	610	270	3	—	0	Embolic type, aortic endo- carditis
12	15-253	51	M	4	172/100	2	3	—	—	375	500	—	—	0	Retinitis
13	19-5	55	M	12	200/120	+	1	—	0 (1 day)	420	520	0	—	1	Retinitis
14	30-415	57	M	3	150/95 180/100	4	2	140 (6 wks.)	2 (10 wks.)	430	360	1	50	1	Edema of retina
15	29-1799	62	M	1	190/110	4	1	190 (2 wks.)	—	—	550	0	48	1	
16	11-77	70	M	—	—	+	3	—	—	475	335	0	—	3	

The percentage of phenolsulphonethalein excreted in 2 hours is shown. Numerals are used to indicate the intensity of albuminuria, edema, passive congestion of the liver and the extent of formation of epithelial crescents. The time before death is indicated in some of the observations. The — sign means no observation.

On admission, Sept. 16, 1937, a severe generalized anasarca was noted. There was dyspnea, with evidence of edema of the lungs and ascites. The temperature was subnormal and the heart rate was rapid and regular. The eyegrounds showed a grayish exudate but the retinal arteries appeared normal. The blood pressure was 192/112 mm. Hg. The total diuresis for 24 hours was only 45 cc. Death occurred on Sept. 18, 1937.

Laboratory Examinations: Hemoglobin 50 per cent; red cell count 2,700,000; leukocytes 12,900—84 per cent neutrophils; blood urea nitrogen, 77.8 mg. per cent; carbon dioxide combining power of the blood, 16 per cent. Plasma proteins: albumin 2.2, globulin 2—total protein 4.2 gm. per cent.

In subacute glomerulonephritis the blood pressure usually rises steadily and is commonly very high in the advanced stages. Disturbances of vision from retinal edema or hemorrhages are frequent.

The urine contains abundant albumin and casts. The specific gravity often decreases, and oliguria is frequently a prominent feature. Hematuria is infrequent.

Edema is usually rather well marked and was not absent in any of our cases. The plasma proteins were determined in only 1 case (Case 2), and their low level is an ample explanation of the edema in this instance. Cardiac failure may have been a contributory cause of edema in Cases 4, 6, 8 and 11, since there was a definite chronic passive congestion of the liver in each of these. In Case 11 the associated heart disease was presumably the cause of the severe passive congestion of the liver. Probably the low level of the plasma proteins is the chief cause of edema, since edema was present in 9 cases in which there was no passive congestion of the liver. A high venous pressure is a more delicate indication of cardiac failure than passive congestion of the liver, but this observation was not available.

Renal insufficiency may always be demonstrated if tests are made in advanced stages of the disease. Death was apparently due to uremia in all of our cases. As in other forms of uremia, the hemoglobin decreases as renal insufficiency develops.

The heart is usually only moderately enlarged. Omitting the 8 year old child (Case 1) and the case of endocarditis (Case 11) the average weight of the heart was 389 gm. Apparently hypertension must be maintained at a high level for a long time before marked cardiac hypertrophy develops.

The kidneys are of normal size or moderately enlarged, but

occasionally they are very large (Case 2). The average combined weight of the kidneys in 15 cases is 380 gm., but if Case 2 be omitted the average weight of the remaining 14 cases is only 356 gm. The external surfaces are smooth and on section the cortices are of normal or increased width. Microscopically, as noted above, there is found a uniform diffuse tubular atrophy (Fig. 1). There are no islands of normal tubules, as in chronic nephritis, and the tubular atrophy is not extreme. Since all the tubules are involved it is obvious that the patient cannot survive to the point of extreme tubular atrophy such as one finds in parts of the cortex in chronic nephritis. The glomeruli are uniformly involved and severely obstructed. There are no solid hyaline glomeruli, since the process is too recent, but there may be small hyaline areas in the centers of some of the glomerular lobules. Epithelial crescents may play a rôle by compressing the glomeruli, but the most important cause of glomerular obstruction is endothelial proliferation.

There are different histological types. In the usual proliferative form the centers of the glomerular lobules consist of a dense mass formed by splitting and fusion of the central capillary basement membranes. This central mass may show beginning hyaline degeneration. The peripheral zones of the glomerular lobules contain narrow capillaries which are, however, insufficient in number and caliber to afford adequate filtration. Decreased glomerular filtration results in partial disuse atrophy of the tubules.

In Cases 1 and 7 the glomerular obstruction was due largely to epithelial crescents. In Cases 10 and 11 the lesions were all of embolic type, and in 1 of these, Case 10, no endocarditis was present. These 2 cases might also be classified as embolic glomerulonephritis, but lesions of embolic type frequently occur independently of endocarditis. Occasionally uremia is due in part to extensive tubular obstruction by casts as in Case 2.

GROUP II. CHRONIC GLOMERULONEPHRITIS IN WHICH DEATH WAS DUE TO AN INTERCURRENT DISEASE (TABLE III)

The 8 cases listed in Table III are examples of chronic glomerulonephritis in which death was caused by another disease before marked renal insufficiency had developed. These cases are of particular interest since little is known of the structural changes

TABLE III

Group II. Chronic Glomerulonephritis in which Death was due to an Intercurrent Disease

Case No.	Autopsy No.	Age yrs.	Sex	Duration of symptoms	Blood pressure mm. Hg.	Albuminuria	Edema	Urea nitrogen mg./100 cc.	Phenolsulpho- naphthalein %	Weight of heart gm.	Weight of kidneys gm.	Passive conges- tion of liver	Hemoglobin %	Hyaline elon- gation	Tubular atrophy	Cause of death
17	24-807	20	F	7 mos.	138/86 158/98	2	1	49.4 (6 mos.) 15.5 (8 days)	47 (5 days)	400	465	—	40	0	0	Streptococcic bacterie- mia
18	31-794	22	F	3 yrs.	140/90 (3 yrs.) 150/90 (5 days) 170/90	4	0	—	61 (3 yrs.)	—	Large	1—	—	10	1—	Tetanus following in- duced abortion
19	33-292	34	F	2 mos. +	110/70	4	1—	28 (2 days)	—	420	462	3	70	10	10	Ulcerative colitis, en- cephalomalacia Cardiac failure
20	27-511	43	F	1 yr.	—	—	1	—	—	538	304 (one)	1	—	—	0	Bronchopneumonia
21	25-146	51	F	4 yrs.	—	—	1	0	29.5 (2 wks.)	240	190	—	—	90	0	Marked emaciation, is- chiofretal abscess Cardiac failure
22	28-1110	58	F	4 yrs. (?)	—	—	1—	3	—	215	195	1—	1—	40	20	Purulent bronchitis, pericarditis
23	31-748	70	F	4 mos. +	170/100	2	2	3	Normal (1 wk.) 15.6 (1 day)	606	438	2	34	58	5	
24	34-1408	80	F	? yr.	138/60	1	1	1	—	450	—	—	—	—	—	

Explanations as in Table II. 1_d = diffuse tubular atrophy of mild degree.

in the kidneys during the long interval between the primary acute attack and the terminal chronic stage.

The diagnosis of glomerulonephritis in this group is based entirely on the microscopic structure of the kidneys except in Cases 17 and 18 in which it was also established clinically. In the other 6 cases the renal symptoms were overshadowed by those of the major illness. In Case 19 the clinical picture was that of ulcerative colitis with severe albuminuria and mild edema. In Case 20 the clinical impression was cardiac failure. In Case 21 there was a history of dyspnea and palpitation for 4 years, and a general anasarca developed during the last month of life. In Case 22 there was an ischiorectal abscess of 4 years duration, as well as severe emaciation. There was only a trace of albumin in the urine and the edema developed terminally. Case 23 was a typical example of hypertension with cardiac failure.

There was a very slight elevation of the blood urea nitrogen in Cases 19 and 21, but there was no clinical or anatomical evidence of serious impairment of renal function in any instance.

The clinical history in Case 18 will be given in detail since this case is a good illustration of the group under discussion. I am indebted to Dr. George Fahr for the excellent clinical record which follows.

The patient, an unmarried woman, 19 years of age, was admitted to the hospital Feb. 15, 1928, complaining of a cold, sore throat, headache and swollen cervical lymph nodes. The diagnosis was acute tonsillitis. On February 27th the urine contained albumin + + + +, the leukocyte count was 13,300, and the blood pressure was 140/90 mm. Hg. The 2 hour excretion of phenolsulphonephthalein was 61 per cent. There was no edema. The diagnosis was acute glomerulonephritis. The patient gradually improved. On March 1st, the leukocyte count was 8700 and the blood pressure on March 7 was 126/84 mm. Hg. Tonsillectomy was performed on March 13, 1928, and the patient was discharged on April 19, 1928.

She was readmitted on Oct. 8, 1928, with symptoms of sinusitis. The urine showed albumin + +; the leukocyte count was 9050; and a concentration-dilution test gave a range of specific gravity from 1005 to 1023. The clinical diagnosis was now chronic glomerulonephritis, which was regarded as the outcome of the attack of acute glomerulonephritis in the preceding February. The patient was discharged after a few days and continued her work as a nurse in apparently good health until the onset of her final illness on May 1, 1931.

She was readmitted to the hospital May 1, 1931, suffering from acute endometritis following an induced abortion. Tetanus developed and death occurred on May 6, 1931. There was albuminuria but no edema. The blood pressure was 150/90 mm. Hg. No functional studies were made at this time.

At postmortem the kidneys were found to be enlarged with smooth external surfaces. Microscopically about 10 per cent of the glomeruli are hyaline, and there is atrophy of their associated tubules. Practically all of the other glomeruli present a similar appearance. They are slightly enlarged and their lobulations are distinct (Fig. 2). Under higher magnification the lobules show solid central portions with small peripherally situated capillaries (Fig. 3). There is some increase of endothelial cells but the capillaries are not markedly constricted. The peripheral capillary basement membranes are not thickened. Glomerular filtration is evidently fairly good since there is no atrophy of the tubules associated with these glomeruli.

The structural changes in the kidneys of the other 7 cases in Group II correspond to the above description aside from minor variations. The kidneys are not contracted but are usually somewhat enlarged. In Case 19 there were some epithelial crescents, the capillary obstruction was more pronounced than is shown in Figures 2 and 3 and had resulted in a slight tubular atrophy. In Case 21 the glomerular lesions were less intense than in Case 18. In Case 23 an arteriolosclerosis was present which was responsible for most of the hyaline glomeruli.

We may now trace the pathogenesis of the glomerular lesion in chronic glomerulonephritis. The normal glomerular lobule is composed of capillaries with a distinct basement membrane in both inner and outer walls (compare Figs. 3 and 4, Plate 130, *Am. J. Path.*, 1936, 12, 801-824). In acute glomerulitis there is an increase of endothelial cells and the central basement membranes of the capillaries are split into numerous irregular fragments which have been called intracapillary fibers. In severe glomerulitis the capillaries are completely obstructed, but in the less severe lesions (Fig. 4), from which the chronic forms probably develop, the capillaries are not closed completely. As the inflammation subsides the blood forces the intracapillary fibers to the center of the lobule where they become fused to form a central hyaline mass and the lobule then has the appearance shown in Figures 3 and 7. If the capillaries are completely closed during the acute attack the glomerulus becomes hyaline. The functioning glomeruli in chronic azotemic glomerulonephritis usually resemble those shown in Figures 2 and 3. The most important difference between the

early or mild lesions of Group II and those of advanced azotemic glomerulonephritis is that in the latter group nearly all the glomeruli have become hyaline; the persistent functioning glomeruli in the advanced stage are not notably different in structure from those of the early stage. One may well believe that the progress from the early to the advanced stage of chronic glomerulonephritis is due to repeated acute attacks which obstruct more and more of the glomerular circulation.

The only publication known to me which deals with the structure of chronic glomerulonephritis in the pre-uremic or latent chronic stage is one by Dorothy Russell² in 1934. She described a kidney removed under an erroneous diagnosis 16 years before death. The remaining kidney at autopsy showed a typical advanced chronic glomerulonephritis. The illustration of the kidney removed 16 years before death is shown only under low magnification but resembles those of Group II.

GROUP III. ADVANCED CHRONIC GLOMERULONEPHRITIS OF THE AZOTEMIC TYPE (TABLES IV, V AND VI)

This group, which comprises 117 cases, has been divided into 3 subgroups in accordance with certain clinical and pathological features. Subgroup A (Table IV) includes 30 cases in which there was a definite history of acute glomerulonephritis. Subgroups B and C (Tables V and VI) include 87 cases in which no history of an acute attack was obtained; in the former the kidneys weighed together 250 gm. or more, in the latter they weighed less than 250 gm. and showed varying degrees of contraction. It will appear that the separation of subgroups B and C has little clinical significance but it will serve to emphasize certain structural differences between large and small kidneys.

Considering Group III as a whole we may call attention to certain features.

Duration: The total course of the disease is known only in the 30 cases listed in Table IV. In this group the duration ranges from 18 months to 26 years. The average time between the acute attack and death is 10 years. The duration is as follows: under 5 years, 8 cases; 5 to 10 years, 9; 10 to 15 years, 5; 15 to 20 years, 5; 20 to 26 years, 3.

The acute attack is often followed by a latent period during

which the patient considers himself well, although it is probable that symptoms and signs of the disease could be detected by careful examination. As shown in the table, the latent period may last many years (Case 54, 24.5 years; Case 46, 23.5 years; Case 39, 14 years). However, the active chronic stage may begin immediately after the acute stage, *e.g.* Cases 29, 32 and 35.

The duration shown in Tables V and VI is merely the length of the active chronic stage in most of the cases. It is measured from the date of onset of symptoms as given by the patient except in a few cases in which it is dated from the finding of albuminuria in the course of an examination for life insurance. It is remarkable that 16 patients worked at their usual occupations and considered themselves in good health up to a period from 1 month to 3 months before death, although the kidneys at postmortem often showed a high degree of contraction indicating a duration of many years. It is clear that the duration of symptoms is far less than the total course of the disease. The average duration in the group in which the complete history is known (Table IV) is 10 years, while in those with no history of acute onset (Tables V and VI) it is only 3 years.

Frehse,³ in a study of 248 cases of nephritis, found that 68 lasted over 5 years, 23 over 10 years, 19 over 15 years, 6 over 20 years, and 3 over 40 years.

The Acute Attack: In most instances the acute attack was typical and fairly severe, confining the patient to bed for a number of weeks, but in some cases it was mild and characterized only by headache with albuminuria or edema. It is easily possible that in the cases with no history of acute glomerulonephritis there was a mild attack that was not recognized as nephritis. For example, in Case 18 the condition following the attack of acute tonsillitis would not have been recognized as acute glomerulonephritis if a careful study had not been made. It is the usual experience that in a majority of the cases first seen in the active chronic stage careful inquiry does not reveal an illness which can be interpreted as acute glomerulonephritis. On the other hand, patients first seen in the acute stage and subsequently followed for a number of years show all the variations in the clinical course that appear in Table IV. Some of them pass directly into the active chronic stage and others remain in fairly good health for a number of years.

TABLE IV

Group III. Su., p A. Chronic Azotemic Glomerulonephritis with a History of Acute Glomerulonephritis

Case No.	Autopsy No.	Age	Sex	Total duration	Duration of active chronic	Initial infection	Blood pressure	Urea nitrogen	Non-protein nitrogen	Phenylsulfonphthalein	Weight of heart	Weight of kidneys	Passive congestion of	Hemoglobin	Edema	Retinitis	Epithelial crescents	Histological type	Comment
25	35-2064	15	M	4	4 yrs.	—	mm. Hg. 220/110	mg./100 cc. —	mg./100 cc. 285 (1 day)	% —	gm. 380	gm. 460	o	% 59	2	—	o	b ₁	
26	36-2149	15	F	1.5	18 mos.	Tonsillitis	148/100 (18 mos.) 170/130	34 (18 mos.) 118 (1 day)	—	49 (18 mos.) 0 (1 day)	450	160	2	54 (18 mos.)	1	—	o	a	Plasma proteins 5.5 gm. %
27	25-246	16	F	4	16 mos.	Sore throat	180/130	70 (1 mo.)	—	0 (1 mo.)	400	163	1—	38	2	+	2	a	
28	23-846	18	M	2	1 mo.	—	200/134	181 (2 days)	—	—	475	245	1—	50	1	+	3	a ₂	
29	31-1974	19	M	9	9 yrs.	Scarlet fever	180/110	110 (1 mo.) 238 (1 day)	—	—	550	200	o	28	1	—	1	a	
30	34-763	19	F	4	4 yrs.	Common cold	180/148 (5 mos.)	—	102 (1 wk.) 218 (1 day)	20 (5 mos.)	350	150	2	46	2	+	2	a	Exacerbations
31	36-1587	20	M	10	2 yrs.	Common cold	190/125 (1 mo.)	—	171 (1 mo.)	—	585	180	o	35	1	+	o	a	
32	33-1180	21	F	14	14 yrs.	Scarlet fever	160/100 (4 mos.)	—	327 (2 wks.)	2 (4 mos.)	325	100	1	35	3	o	3	a	
33	28-170	21	M	3	3 yrs.	—	230/160	28 (1 yr.) 119 (4 days)	—	2 (4 days)	420	174	1	50	2	—	1	a	
34	32-2024	24	F	8	5 yrs.	—	195/140	200 (2 days)	—	0 (6 wks.)	475	210	1—	40	2	+	o	a	Exacerbations
35	27-452	24	M	18	18 yrs.	—	158/80	97 (3 wks.)	—	0 (3 wks.)	320	135	—	16	o	+	3	a	
36	23-48	25	M	9	9 yrs.	Measles	210/140	165 (4 days)	—	4 (1 mo.)	—	163	—	37	o	+	1	a	
37	17-62	27	F	8	8 yrs.	—	160/? (8 yrs.) 185/100 (2 wks.)	101 (2 wks.)	—	0 (2 wks.)	455	150	o	40	1	Pv	2	a	

25 27-43-
26 23-48
27 17-62

25 M 0 0 yrs.
27 F 14 14 yrs.

Go/F
(182/100)
(2 wks.)

(2 wks.)

38	19-264	29	M	6	2 mos.	Common cold	170/80	87 (1 mo.)	—	—	0 (1 mo.)	435	340	1	25	1	+	2	b ₁
39	36-2304	29	M	14	1 mo.	Scarlet fever	170/110	27 (1 mo.)	—	—	0 (1 mo.)	540	80 (one)	0	—	0	+	0	a
40	27-261	31	M	3	3 yrs.	Sore throat	180/95 (3 yrs.) 180/90	260 (2 wks.)	—	—	—	420	175	1	45	0	+	0	a
41	30-1480	32	F	16	11 yrs.	—	142/82 (11 yrs.) 250/130	50 (1 wk.)	—	—	60 (10 yrs.) 0 (1 wk.)	350	100	0	75	0	—	0	a
42	18-117	32	M	10	2 mos.	—	190/140	—	—	—	0 (2 wks.)	630	405	2	—	2	—	2	bd
43	33-2022	33	M	12	3 mos.	—	220/130	280 (3 days)	—	—	—	550	180	1	—	1	+	0	a
44	34-267	35	M	16	10 yrs.	Influenza	180/100	—	328 (3 wks.)	—	—	575	190	0	36	0	+	0	a
45	27-49	35	F	6	5 yrs.	—	148/90 (3 days)	101 (1 day)	—	—	—	300	Small	0	30	0	0	2	a
46	28-1249	36	M	24	5 mos.	—	236/160	152 (6 wks.)	—	—	0 (7 wks.)	420	90	0	26	0	—	1	a
47	31-1746	37	M	15	12 yrs.	—	220/130	190 (10 days)	—	—	5 (2 wks.)	680	225	0	39	0	+	1	ad
48	31-241	38	F	23	16 yrs.	Scarlet fever	225/135	80 (5 days)	—	—	4 (7 days)	430	220	1	63	1	0	0	ad
49	35-562	40	M	9	—	—	134/80 (3 days)	279 (1 day)	—	—	—	546	177	0	36	0	0	2	a
50	27-653	43	M	6	5.5 yrs.	Sore throat	128/80 (6 yrs.) 260/120 (1 mo.)	15 (6 yrs.) 150 (2 days)	—	—	—	700	195	1	—	2	p _v	0	a
51	31-1146	44	F	4	4 yrs.	—	—	65 (5 days)	—	—	0 (5 days)	350	200	0	—	0	—	1	a
52	37-1993	46	F	15	15 yrs.	—	140/90 (5 yrs.) 215/140 (3 mos.) 182/106 (1 mo.) 238/124	31 (5 yrs.) 191 (2 wks.) — 47.6 (6 mos.) 74 (2 days)	—	—	62 (5 yrs.) 23 (3 mos.) — 70 (2 mos.) 25 (2 mos.)	380	130	—	72	1	—	0	ad
53	33-867	53	F	5	5 yrs.	Common cold	182/106 (1 mo.)	—	163.8 (3 wks.)	—	—	450	140	0	54	0	0	2	a
54	30-1826	52	M	26	1.5 yrs.	Tonsillitis	238/124	—	—	—	—	627	254	0	55	0	—	0	b ₁

Explanations as in Table II. P_v = poor vision.

TABLE IV

Group III. Subgroup A. Chronic Azotemic Glomerulonephritis with a History of Acute Glomerulonephritis

Case No.	Autopsy No.	Age yrs.	Sex	Total duration yrs.	Duration of active chronic	Initial infection	Blood pressure mm. Hg.	Urea nitrogen mg./100 cc.	Non-protein nitrogen mg./100 cc.	Phenolsulpho- naphthalein %	Weight of heart gm.	Weight of kidneys gm.	Passive con- gestion of liver	Hemoglobin %	Edema	Retinitis	Epithelial crescents	Histological type	Comment
25	35-2064	15	M	4	4 yrs.	—	220/110	—	285 (1 day)	—	380	460	0	59	2	—	0	b ₁	
26	36-2149	15	F	1.5	18 mos.	Tonsillitis	148/100 (18 mos.) 170/130	34 (18 mos.) 118 (1 day)	—	49 (18 mos.) 0 (1 day)	450	160	2	54 (18 mos.)	1	—	0	a	Plasma pro- teins 5.5 gm. %
27	25-246	16	F	4	16 mos.	Sore throat	180/130	70 (1 mo.)	—	0 (1 mo.)	400	163	1—	38	2	+	2	a	
28	23-846	18	M	2	1 mo.	—	200/134	181 (2 days)	—	—	475	245	1—	50	1	+	3	a ₂	
29	31-1074	19	M	9	9 yrs.	Scarlet fever	180/110	110 (1 mo.) 238 (1 day)	—	—	550	200	0	28	1	—	1	a	
30	34-763	19	F	4	4 yrs.	Common cold	180/148 (5 mos.)	—	102 (1 wk.) 218 (1 day)	20 (5 mos.)	350	150	2	46	2	+	2	a	Exacerba- tions
31	36-1587	20	M	10	2 yrs.	Common cold	190/125 (1 mo.)	—	171 (1 mo.)	—	585	180	0	35	1	+	0	a	
32	33-1180	21	F	14	14 yrs.	Scarlet fever	160/100 (4 mos.)	—	327 (2 wks.)	2 (4 mos.) 2 (4 days)	325	100	1	35	2	0	3	a	
33	28-170	21	M	3	3 yrs.	—	230/160	28 (1 yr.) 119 (4 days)	—	—	420	174	1	50	2	—	1	a	
34	32-2024	24	F	8	5 yrs.	—	195/140	200 (2 days)	—	0 (6 wks.)	475	210	1—	40	2	+	0	a	Exacerba- tions
35	27-452	24	M	18	18 yrs.	—	158/80	97 (3 wks.)	—	0 (3 wks.)	320	135	—	16	0	+	3	a	
36	23-48	25	M	9	9 yrs.	Measles	210/140	165 (4 days)	—	4 (1 mo.)	—	163	—	37	0	+	1	a	
37	17-62	27	F	8	8 yrs.	—	160/? (8 yrs.) 185/100 (2 wks.)	101 (2 wks.)	—	0 (2 wks.)	455	150	0	40	1	Pv	2	a	

TABLE V

Group III. Subgroup B. Chronic Glomerulonephritis of the Azotemic Type. No History of Acute Glomerulonephritis. Kidneys Weighing Together 250 gm. or More

Case No.	Autopsy No.	Age	Sex	Duration of symptoms	Blood pressure	Urea nitrogen	Non-protein nitrogen	Phenolsulpho-nephthalein	Weight of heart	Weight of kidneys	Passive con-gestion of liver	Hemoglobin	Edema	Retinitis	Epithelial crescents	Hyaline glomeruli	Histological type	Comment	
55	33-1854	8 yrs.	F	1 yr.	mm. Hg. 210/120	—	mg./100 cc. 68 (8 days) 156 (1 day)	% —	gm. —	gm. 80	—	% —	0	—	1	20	be		
56	33-855	17	M	8 yrs.	208/130	139 (2 days)	—	—	505	345	—	—	0	+	0	50	be		
57	34-2188	18	F	4 yrs.	140/100 (5 days) 128/60 (5 days)	39 (5 days)	—	—	275	300	0	—	1	—	0	40	b ₁		
58	25-171	19	M	2 mos.	190/154 (5 days)	—	—	—	360	325	—	—	0	—	1	50	b		
59	22-118	20	F	3 yrs.	200/130 (6 mos.) 235/130	76.6 (6 days)	—	0 (6 days)	420	400	2	70	1 3	+	3	20	b ₁		Exacerba-tions Scarlet fever at age of 6 yrs.
60	36-2331	21	M	6 mos. +	—	—	—	—	525	325	1	—	0	+	0	90	b		
61	34-2102	22	F	5 mos. +	—	17.4 (5 mos.)	—	20 (2 mos.)	—	300	0	70	1	—	0	0	be		
62	35-1131	24	F	1 yr.	164/92	—	222 (3 days)	—	400	350	2	34	1	—	1	60	b		
63	22-574	29	M	10 mos.	185/125	71 (2 wks.) 142 (2 days)	—	4 (2 wks.)	370	290	0	56	1 0	—	2	20	b ₁		
64	14-192	30	M	—	192/?	—	—	—	490	360	—	—	1	—	1	30	b ₁		
65	33-5	30	M	10 yrs.	115/70 (3 yrs.) 170/110	—	176 (4 days)	0 (2 days)	550	400	0	60	0	+	1	70	b		
66	34-356	31	F	4 yrs.	170/90	—	267 (4 days)	—	570	280	1	94	0	+	2	70	b		
67	10-145	32	M	—	—	—	—	—	530	314	3	—	0 2	—	0	10	b ₁		

TABLE VI

Group III. Subgroup C. Chronic Glomerulonephritis of the Azotemic Type. Kidneys Weighing Together Less than 250 gm.

Case No.	Autopsy No.	Age	Sex	Duration of symptoms	Blood pressure	Urea nitrogen mg./100 cc.	Non-protein nitrogen mg./100 cc.	Phenylsulpho- nephthalein %	Weight of heart gm.	Weight of kidneys gm.	Passive con- gestion of liver	Hemoglobin %	Edema	Retinitis	Epithelial crescents	Histological type	Comment
88	30-519	14	F	1 yr. +	—	176 (3 wks.)	—	—	170	185	0	43	1	—	2	b ₁	
89	15-373	23	M	3 mos.	180/115	—	—	11 (2 mos.)	557	Small	—	50	1	—	0	a	
90	16-384	24	M	3 mos.	170/90	131 (5 days)	—	0 (6 days)	415	205	0	20	1	—	2	b ₁	
91	33-214	24	M	1 mo.	188/90	296 (1 day)	—	—	400	87	0	20	1	—	3	a	
92	32-947	25	M	—	150/70	240 (1 day)	—	—	380	140	0	54	0	—	0	a	
93	37-1501	25	M	6 wks. +	168/98	120	—	0 (2 wks.)	450	122	—	36	1	0	0	a	
94	27-1287	25	M	3 mos.	220/160	82 (2 wks.) 116 (1 day)	—	16 (2 wks.)	325	105	0	65	2	ea	0	a	
95	19-2	25	M	9 mos.	210/118	72 (9 days)	—	0 (9 days)	500	195	—	—	0	+	1	a	Exacer- bations
96	26-286	26	F	4 yrs.	160/110 (3 yrs.) 210/130 (1 yr.)	72 (3 yrs.)	—	—	380	210	2	—	1 0	0	1	b ₁	
97	22-47	27	M	2.5 yrs.	182/110	133	—	0	540	110	0	30	1 0	—	2	a	
98	24-697	27	M	—	122/44 (1 day)	—	—	—	200	52	0	—	0	—	0	a ₁	
99	32-1935	27	M	10 yrs.	194/114	131 (3 wks.) 216 (3 days)	—	—	550	175	1	—	0	+	4	a	

TABLE VI (Continued)

Case No.	Autopsy No.	Age	Sex	Duration of symptoms	Blood pressure	Urea nitrogen	Non-protein nitrogen	Phenylsulpho-naphthalein	Weight of heart	Weight of kidneys	Passive congestion of liver	Hemoglobin	Edema	Retinitis	Epithelial crescents	Histological type	Comment
116	23-293	35 yrs.	M	4 yrs.	mm. Hg. 142/100 (4 yrs.)	mg./100 cc. 120 (2 days)	—	% 0 (3 wks.)	gm. 500	gm. 123	—	% 38	1 0	—	0	a	
117	26-251	35	F	—	202/132 250/160	169 (1 day)	—	—	370	200	1	67	0	+	1	ad	Diabetes
118	31-1197	37	F	3-5 yrs.	158/98 (3 yrs.) 200/120	Normal (3 yrs.) 110 (7 mos.)	—	40 (3 mos.)	350	140	0	74	0	pa	0	a	
119	28-734	37	M	1 yr.	160/130	132 (6 days)	—	0 (6 days)	450	200	1	64	0 2	+	0	a	
120	35-360	37	F	7 yrs.	275/150	31.5 (2 yrs.) 119.7 (1 day)	—	—	544	155	0	47	1—	pv	0	b	
121	28-347	39	F	—	—	37.8 (6 mos.)	—	—	370	226	1	55	1	—	3	b	
122	37-1290	40	M	—	—	62 (1 day)	132 (1 day)	—	300	60	0	—	0	—	0	a	
123	31-1063	40	F	4 yrs.	240/150	155 (2 mos.)	—	53 (2 yrs.) 1 (2 mos.)	520	140	—	44	2	—	2	a	
124	37-643	40	M	2 yrs. +	200/145	—	—	—	600	185	1	50	0	+	0	a	
125	30-1631	41	M	3 yrs.	220/150	—	90 (8 mos.)	—	550	210	—	—	2 0 2	+	1	a	
126	21-434	41	F	1 yr.	180/70	238 (6 days)	—	0 (11 days)	560	150	1—	—	1 1	pv	1—	a	
127	23-604	41	F	7 mos.	210/140	106 (10 days)	—	10 (3 wks.)	385	110	1—	48	3 1	pv	0	a	

The symptoms in the active chronic stage vary in intensity in the different patients.

In 7 cases there was a history of repeated acute exacerbations during which all the symptoms, including edema, albuminuria and decreased renal function became more intense. After the subsidence of the acute exacerbations the symptoms return to their previous levels, but there is a tendency to progressive impairment of renal function. The active chronic stage is therefore characterized either by continuous symptoms of low intensity or by acute exacerbations separated by intervals of varying length during which the symptoms are of only moderate severity.

The Blood Pressure: In the tables the maximum blood pressure is recorded and this pertains to the terminal stages of the disease unless otherwise stated. In a few instances a blood pressure taken some months or years before death is recorded, the time prior to death being indicated in the table. There are 9 cases in which the maximum systolic blood pressure recorded was below 140 mm. Hg., viz., Cases 49, 58, 79, 98, 107, 109, 114, 129 and 131. It is unlikely, however, that all of these cases represent chronic glomerulonephritis without hypertension. In Cases 49, 79 and 114 the marked enlargement of the heart is strong evidence that hypertension was present at some previous period, and in Cases 58, 98, 107, 109 and 131 the blood pressure was not recorded until shortly before death, a period during which terminal circulatory failure often develops. The period of observation in all of these cases is too short to justify the diagnosis of "chronic glomerulonephritis without hypertension." However, the small size of the heart in Cases 57, 98 and 138 suggests that hypertension did not play an important rôle in these instances. Cases have also been reported in which there was no elevation of blood pressure during a long period of observation (Bannick ⁴).

In 88 cases with a maximum systolic pressure of 150 mm. Hg. or higher the distribution was as follows: 150 to 170 mm. Hg., 12 cases; 170 to 200 mm. Hg., 39 cases; over 200 mm. Hg., 37 cases. The large proportion of cases (30 per cent) with a systolic pressure above 200 mm. Hg. is surprising. In one instance a pressure of 275 mm. Hg. was recorded. This is conclusive evidence that a blood pressure above 200 mm. Hg. is not evidence against a diagnosis of chronic glomerulonephritis as is sometimes supposed.

The blood pressure usually tends to rise to higher levels as the renal disease progresses (note Cases 26, 41, 50, 52, 65, 69, 96, 103, 116, 118 and 128). Over a period of years it rises gradually to a maximum and usually does not decrease until a short time before death. The blood pressure rises as renal insufficiency increases. This phenomenon is in striking contrast with primary hypertension in which high levels of blood pressure are attained early in the disease.

The Weight of the Heart: The weight of the heart in 111 cases is as follows: 200 to 300 gm., 4 cases; 300 to 400 gm., 30 cases; 400 to 500 gm., 35 cases; 500 to 600 gm., 28 cases; and 600 to 700 gm., 13 cases. The average weight of 110 hearts is 456 gm. The hypertrophy in all instances is of left ventricular type. There is no obvious explanation of the great variation in the size of the heart. Apparently the weight of the heart is not directly related to the duration of the disease nor to the height of the recorded blood pressure. Perhaps we should not expect to find such a correlation since the work required of the left ventricle must depend upon the constancy as well as the degree of hypertension and the length of time involved. It often happens that the blood pressure is only moderately elevated for a number of years and becomes very high only in the terminal stages.

There is some relation between the size of the heart and the age of the patient. In 68 individuals under 40 years of age the average weight of the heart is 438 gm., while in 42 individuals over 40 years of age the average weight is 484 gm. Hearts weighing 500 gm. or more were found in 20 of 68 persons under 40 years of age (30 per cent), and in 20 of 42 over 40 years of age (48 per cent).

The average weight of 68 hearts from males is 489 gm., and of 42 hearts from females 403 gm. The normal heart averages about 50 gm. heavier in males than in females.

Judged by passive congestion of the liver there is some degree of heart failure in nearly one-half of the cases. As shown in Tables IV, V and VI, in 93 cases in which the liver was examined microscopically there were 51 cases with no passive congestion (0), 13 with very slight congestion (1-), 17 with definite but slight central atrophy (1), 9 with some central necrosis (2), and 3 with severe central necrosis (3) such as one finds in death from chronic myocardial failure.

In 1 of the 3 cases with severe passive congestion of the liver (Case 133) a high degree of renal insufficiency was established clinically and in the other 2 an anatomical diagnosis of uremia is justifiable. In 7 of the 9 cases with Grade 2 passive congestion of the liver, uremia was established clinically. We may conclude that heart failure of an appreciable degree may develop in chronic glomerulonephritis and that it may be exceptionally an important contributory cause of death, but uremia is practically always present at the time of death.

In striking contrast with primary hypertension a history of stroke was obtained in only 1 of the 117 patients (Case 78). There was only one typical example of coronary disease (Case 87, a woman 68 yrs. old). In 10 other cases there was complaint of precordial pain at times, but no severe coronary disease was found at postmortem.

Renal Function: It is noteworthy that the blood urea nitrogen seldom rises to high levels until a few weeks before death. Determinations made 6 months or more before death usually range from 25 to 50 mg. per cent. A very marked increase usually takes place during the last 1 or 2 days, and a striking increase is noted during the last 1 or 2 weeks. After the blood urea nitrogen has reached a level of 100 mg. per cent the patient seldom survives more than a few weeks. The marked variations in the terminal level of blood urea in different individuals may be due in part to differences in the amount of protein consumed. It is clear that extreme reduction of the number of functioning nephrons has usually taken place before the blood urea nitrogen rises above 40 mg. per cent. There is commonly a slowly progressive rise of blood urea until the terminal stages when a rapid increase occurs, but occasionally a patient will exhibit a marked elevation, due to an acute exacerbation of the nephritis, which returns to the previous level after the acute process has subsided.

Wakefield and Keith⁵ reported a patient with a blood urea of 290 mg. per cent and a phenolsulphonephthalein output of 0, who recovered and was working in comfort 1 year later.

The phenolsulphonephthalein test gives about the same information as the determination of blood urea. With few exceptions the two tests run parallel, and the only advantage of doing both is the avoidance of possible errors.

Anemia: A hypochromic anemia of fairly severe degree is present in the terminal stages in the great majority of cases. The hemoglobin percentage was 50 or less in over two-thirds of the cases in which it was determined. In only 2 cases was there no anemia. In general the severity of the anemia increases as renal function decreases.

Retinitis: Retinitis, in the sense of retinal hemorrhages and exudates, was observed in 35 of the 46 cases in which the eyegrounds were examined (Table VII) and it was noted in 7 other cases that the patient had poor vision. The data on the caliber of the retinal arteries is too inadequate for discussion. It is well

TABLE VII

*The Relation between Retinitis and the Level of the Systolic Blood Pressure.
(The Eyegrounds Were Examined in Only 46 of the 103 Cases)*

Blood pressure. Systolic mm. Hg.	Number	Retinitis +	Retinitis —
Below 140.....	8	0	1
140-150.....	7	0	3
150-170.....	12	2	2
170-200.....	39	16	2
Above 200	37	17	3
	<hr/> 103	<hr/> 35	<hr/> 11

recognized that retinitis is a result of hypertension. In Table VII it appears that retinitis was present in 33 of 38 patients with a systolic pressure above 170 mm. Hg. In the stage of uremia, primary hypertension and chronic glomerulonephritis usually cannot be distinguished by the appearance of the eyegrounds.

Edema: It is impossible to give a true picture of the degree and duration of edema by means of a summarizing table. In Tables IV, V and VI the numerals give only a rough estimate of the degree of edema during the period of observation. Edema is usually much more marked during acute exacerbations, and it varies greatly in intensity from time to time during the active chronic stage. It was not continuously severe in any of the cases in Group III (Cases 25 to 141). The cases in which edema was a constant and prominent feature are listed in Tables VII, VIII and IX. In 31 patients there was no edema at any time during the period of observation. Edema is often present early in the disease and absent in the

terminal stages, but frequently it is present only toward the end of the illness.

Edema is dependent upon several factors, the most important of which is probably the level of the plasma proteins, but the data in our records are inadequate for a discussion of this relationship. There is no evident relation between edema and the degree of contraction of the kidneys in Group III. Presumably increased venous pressure resulting from myocardial failure is often a factor of importance, but if passive congestion of the liver is taken as evidence of cardiac decompensation it may readily be seen from the tables that there is no close correspondence between edema and cardiac failure. However, there is a possible correlation between terminal edema and passive congestion of the liver. Congestion of the liver was present in 11 of 45 cases without terminal edema and in 18 of 49 cases with terminal edema.

The Relation between the Size of the Kidneys and the Clinical Features: For purposes of comparison the cases without a history of acute onset have been arranged in two groups, *viz.*, those with kidneys weighing 250 gm. or more (Table V), and those with definitely contracted kidneys weighing from 50 to 250 gm. (Table VI). The weights of the kidneys in the group with a history of acute onset (Table IV) may also be compared. It appears from these tables that it is not possible to predict the size of the kidneys from a study of the clinical history. One might expect that the cases of longer duration would show the greater degree of contraction of the kidneys, but this relationship does not obtain as may be seen by a study of Table IV. There is likewise no evident relation between the degree of contraction of the kidneys and the size of the heart, the level of the blood pressure or the prominence of edema. However, when edema is continuously severe, as in Group IV, one may predict that the kidneys will not be contracted.

The size of the kidneys in the azotemic and hydropic types is shown in Chart 1. It appears that the azotemic kidneys are usually much smaller than the hydropic but that some overlapping occurs. In the azotemic type (Group III) the combined weight of the kidneys is as follows: 50 to 99 gm., 13 cases, 11.5 per cent; 100 to 149 gm., 22 cases, 19.5 per cent; 150 to 199 gm., 26 cases, 23 per cent; 200 to 249 gm., 17 cases, 15 per cent; 250 to 299 gm., 10 cases, 8.8 per cent; 300 to 349 gm., 13 cases, 11.5 per cent;

350 to 399 gm., 4 cases, 3.5 per cent; 400 to 449 gm., 3 cases, 2.6 per cent; and 450 to 500 gm., 4 cases, 3.5 per cent. In the hydropic type (Group IV) the kidneys weighed over 300 gm. in 22 of 38 cases, as may be seen in Chart 1. In the azotemic group, in which uremia was always present at death, it may seem remarkable that uremia should develop in some instances when the kidneys are still of normal size and in others not until their weight is less than 100 gm., but these differences are explainable on the basis of the histological structure of the kidneys.

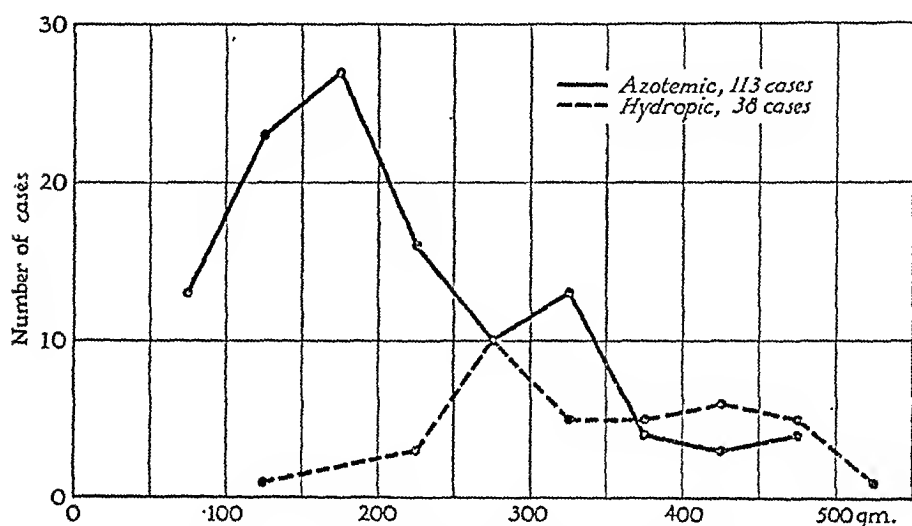


Chart 1. Size of the kidneys in azotemic and in hydropic glomerulonephritis.

THE STRUCTURAL CHANGES IN THE KIDNEYS

The variations in the size of the kidneys in the terminal stages of chronic glomerulonephritis are related to the structural changes that have taken place. These changes may be described as histological types and are so indicated in Tables IV, V and VI. From a study of the histological structure it may be determined why some kidneys are much smaller than others when the stage of uremia is reached.

As a result of acute glomerulitis the glomerular capillaries show a variety of effects ranging from no obstruction at all to complete obliteration. When the capillaries are normal, or only slightly narrowed, glomerular filtration continues and the tubules are unaffected, but when the capillaries are completely closed the glomerulus becomes hyaline and the tubules disappear entirely or

become small epithelial cords. Intermediate degrees of capillary obliteration, however, result in partial but not complete suppression of glomerular function and the associated tubule shows a degree of atrophy corresponding to the state of its glomerulus. Damaged glomeruli with partial tubular atrophy are a prominent feature of many kidneys (Fig. 6). When a large proportion of the glomeruli are of this type the kidneys may reach the stage of renal insufficiency without having undergone contraction. The varying sizes of the kidneys in the stage of uremia depend largely upon the proportion of glomeruli with partial tubular atrophy. In order to condense the histological descriptions the histological type of each kidney is given in the table. These types will be described and illustrated.

1. *Type a*: This is the most common form of chronic glomerulonephritis. The kidneys are small and contracted, the combined weight is usually less than 200 gm. and never over 250 gm. Sometimes they are extremely small (Cases 102, 107, 122 and 128). The cortices are thin. In microscopic sections (Fig. 5) it is noted that a large majority of the glomeruli are hyaline and that the tubules associated with these glomeruli have almost completely disappeared. A differential count usually shows that 80 to 90 per cent of the visible glomeruli are hyaline. The amount of destruction of the cortex is probably even greater than 80 or 90 per cent, since it is known that many hyaline glomeruli are ultimately removed by phagocytes (Moritz and Hayman⁶). Of the persistent glomeruli a few are normal with full sized tubules and some are partially obstructed with tubules showing corresponding stages of atrophy. The proportion of normal to partially obstructed glomeruli varies in different kidneys, sometimes the one predominating, sometimes the other.

Type a₁: This subgroup of Type *a* is represented by only 2 cases (Cases 98 and 103). In both of these the kidneys were very small but only a few hyaline glomeruli were to be seen. It is assumed that in these cases the hyaline glomeruli have been absorbed. One of these (Case 98) was a dwarf, weighing 80 pounds, and it is conceivable that this represents primary hypoplasia rather than atrophy.

Type a₂: This is represented by only 1 case (Case 28). There is a terminal acute glomerulonephritis superimposed on a chronic

glomerulonephritis. The persistent glomeruli show fresh epithelial crescents and other acute changes.

Type ad refers to Type *a* with an associated arteriolosclerosis. There are 8 cases with arteriolosclerosis (Types *ad* and *bd*) and all are characterized by very high blood pressure. The arteriolosclerosis is diffuse and severe. It is not uncommon to find an occasional hyaline arteriole in this disease but severe arteriolosclerosis is unusual.

Many investigators have apparently confused atrophy of small arteries and arterioles with true arteriolosclerosis. Segments of the cortex which contain nothing but hyaline glomeruli and atrophic tubules are functionless and require no blood. The arteries and arterioles supplying such scar-like areas undergo a disuse atrophy which may readily be confused with arteriolosclerosis, but the change is chiefly medial fibrosis and not intimal disease. Arteriolar disease, apart from atrophy, is so rare in chronic glomerulonephritis that it may be an accidental relationship. The structural changes in the arterioles in chronic glomerulonephritis do not support the argument that hypertension causes arteriolosclerosis.

2. *Type b (Fig. 6)*: In this group the kidneys may show a slight reduction in size, a normal weight or even an enlargement. The size of the kidneys is not directly related to the duration of the disease. On microscopic examination it is found that the hyaline forms constitute less than one-half of the visible glomeruli. Frequently only 10 to 20 per cent of the glomeruli are hyaline, and rarely no hyaline glomeruli are to be seen. The most frequent type of nephron in these large kidneys is a damaged glomerulus with moderate atrophy of its tubule. With this type of lesion there is not much shrinkage of the renal cortex.

Type b₁ is a subgroup of Type *b* in which there are few or no normal glomeruli and tubules, the great majority of the nephrons being partially obstructed glomeruli with varying degrees of tubular atrophy (Fig. 7).

Type bd refers to Type *b* with an associated arteriolosclerosis; and Type *be* indicates Type *b* with extensive thrombosis of arterioles. There are 3 cases with widespread acute thrombosis of arterioles.

It is evident from the foregoing descriptions that small kidneys are those in which a large proportion of the nephrons have under-

gone complete atrophy because of complete closure of all the glomerular capillaries. In the small kidneys there are nearly always a few normal glomeruli with normal sized or hypertrophic tubules, and there are usually some partially closed glomeruli with tubules of diminished size. In the large kidneys normal and injured glomeruli outnumber the hyaline forms. Damaged glomeruli have a reduced functional capacity and one normal glomerulus is probably equivalent functionally to several injured forms. In a few instances nearly all the functioning glomeruli are of the damaged type (Type *b*₁). Uremia evidently may develop, as it does in the subacute type, before a large proportion of the glomeruli have become hyaline.

It is unlikely that the complex structure of the kidney seen at postmortem is the result of a single acute attack. In acute glomerulonephritis the intensity of the injury varies in different glomeruli—some escape with only minor injury, others suffer occlusion of a part of the glomerular circulation, and some exhibit complete capillary occlusion. In the healing stage after such an acute attack we would expect to see some normal glomeruli and tubules, some partially obstructed glomeruli with moderate atrophy of their tubules, and some hyaline glomeruli with disappearance of tubules. After the acute stage has subsided we would expect renal function to continue at a constant but reduced level. It is probable that repeated reinfections are responsible for the progressive failure of renal function in chronic nephritis.

GROUP IV. CHRONIC GLOMERULONEPHRITIS OF THE HYDROPIc TYPE

In all the cases of this group edema was present during the greater part of the course of the disease and usually it was a very prominent feature. There were often one or more acute exacerbations during which albuminuria and edema were very severe, and remissions during which these features were much less intense. From the clinical standpoint all three subgroups of Group IV may be regarded as lipid nephrosis in the sense in which this term is now generally used. There are no clinical features by which the three subgroups may be distinguished from one another, but there are histological differences in the structure of the glomeruli.

Subgroup A (Table VIII): In this group there are 6 cases that

TABLE VIII

Group IV. Subgroup A. Hydropic Type. Glomerular Structure of Proliferative Type

Case No.	Autopsy No.	Age	Sex	Duration of symptoms	Albuminuria	Edema	Blood pressure	Urea nitrogen	Non-protein nitrogen	Phenolsulpho- naphthalein	Cholesterol	Plasma proteins	Weight of heart	Weight of kidneys	Hemoglobin	Passive congestion of liver	Hyaline glomeruli	Tubular atrophy	Basement membrane	Cause of death	
142	37-524	16 yrs.	F	14 mos.	4	4	mm. Hg. 145/90 (1 yr.) 220/150	mg./100 cc. 15.4 25.2 (1 mo.)	mg./100 cc. —	% 44 (1 yr.) 12 (1 wk.) 17 (2 wks.)	—	gm. % 4.54 (1 yr.) 1.76 (1 mo.) —	gm. 498	gm. 340	% 46	0	0	10	rp	0	Hydrothorax
143	34-2212	21	F	9 mos.	1 2	4	140/86 (4 mos.) 120/70 (3 wks.)	22.4 (3 wks.)	—	—	—	—	358	402	50	—	—	0	0	0	Parotitis
144	34-1543	41	M	7 yrs.	4	4	190/130	—	—	—	—	—	325	435	—	0	10	rp	0	0	Hydrothorax and ascites
145	24-580	68	M	4 mos.	2 4	3	200/115	41.5 (1 mo.)	—	—	—	—	350	275	—	1	0	0	rp	rp	Lobar pneumonia
146	28-906	37	F	3.5 yrs.	3	1	142/104 162/114	38.5 (3 mos.)	—	—	—	—	340	136	54	3	50	2	2p	2p	Mitral stenosis
147	34-633	76	F	3 mos.	1	4	—	—	—	—	—	—	375	240	20	1	0	0	2p	2p	Severe anemia

correspond clinically to lipoid nephrosis but belong anatomically with proliferative glomerulonephritis. A representative case of this group is reported fully:

CASE 142. Clinical History: A white female, 16 years old, was first admitted to the hospital Jan. 25, 1936. On Dec. 24, 1935, she had had several short attacks of pain in the right upper quadrant associated with flatulence and belching. During the next few days there were repeated attacks of vomiting but no pain. About Jan. 2, 1936, she first noticed swelling of the face, feet and ankles. The edema disappeared after a few days in bed. She had had scarlet fever about 1 year before the onset of the present illness and an occasional attack of sore throat during the previous 2 years.

On admission, Jan. 25, 1936, there was a marked edema of the face and the extremities. The systolic blood pressure was 145 and the diastolic 90 mm. Hg. Rales were heard in the bases of both lungs. The fundi were normal. Repeated examinations of the urine showed a specific gravity from 1017 to 1031, albumin + + + +, and many casts and erythrocytes in all specimens. The 24 hour diuresis varied from 200 cc. to 1700 cc., being usually about 700 cc. The fluid intake was about 1200 cc.

The hemoglobin fell from 66 per cent on admission to 46 per cent shortly before death, and the erythrocytes from 3,660,000 to 1,800,000. The 2-hour excretion of phenolsulphonephthalein was 44 per cent (February 1936), 12 per cent (March 1936), and 26 per cent (October 1936). The blood urea nitrogen varied from 15.4 to 25.2 mg. per cent, creatinin from 1.5 to 2 mg. per cent. The total plasma proteins were 4.54 gm. per cent (April 1936), 6.27 gm. (May 1936) and 1.76 gm. (February 4, 1937).

The blood pressure varied from 145/90 on admission to 220/150 in November 1936. The edema varied in intensity from time to time but was usually quite pronounced. Dyspnea was usually a prominent symptom and headache was often severe. Death occurred on March 3, 1937. The clinical diagnosis was lipoid nephrosis.

At postmortem there was extreme anasarca. The peritoneal cavity contained about 1000 cc. of clear fluid, the right pleural cavity 500 cc., the left pleural cavity 800 cc., and the pericardial cavity 300 cc. There was also marked edema of the lungs. Death was apparently due largely to edema of the lungs and hydrothorax.

The heart weighed 498 gm. and showed left ventricular hypertrophy. There was no passive congestion of the liver.

The kidneys weighed 340 gm. and showed smooth external surfaces. On section a yellowish tinge was noted. On microscopic examination only an occasional hyaline glomerulus is noted, and there is a very little patchy tubular atrophy. All the glomeruli are moderately enlarged and uniformly involved. Lobulation is distinct. The lobules show central masses of hyaline of varying amount, formed by thickening and fusion of the centrally placed

capillary basement membranes (Fig. 8). The glomerular structure corresponds entirely to that of chronic proliferative glomerulonephritis, but death occurred from edema before any appreciable atrophy of parenchyma had taken place. The structure is therefore quite different from that of the great majority of cases in which death is due to uremia and the kidneys are atrophic. There is no diffuse thickening of the capillary basement membranes which characterizes most cases of lipoid nephrosis.

The other 5 cases of Subgroup A are similarly examples of chronic proliferative glomerulonephritis in which death occurred in a comparatively early stage of the disease. It may be said therefore that chronic proliferative glomerulonephritis may in unusual instances completely reproduce the clinical syndrome which we are accustomed to call "lipoid nephrosis."

Subgroup B (Table IX): This group includes 9 cases in which the clinical diagnosis was lipoid nephrosis. On the basis of the data presented a few of these cases may be considered examples of pure lipoid nephrosis in the broad sense but there was in most instances a little hypertension, some azotemia or a reduced output of phenolsulphonephthalein. There was only one death from uremia. In one instance, Case 156, edema was not very prominent and the case was classified in this group because of the thick capillary basement membranes. In Case 148 there was marked cardiac hypertrophy and hypertension. The plasma proteins were very low in the 2 cases in which they were determined, Cases 149 and 150. There is no evidence in any case that edema was of cardiac origin.

The most interesting feature of this group is the glomerular lesion. In only one instance, Case 148, is there any large proportion of hyaline glomeruli, and in 2 cases there are none. Aside from the hyaline forms the glomeruli are nearly all large with many permeable capillaries.

The glomerular lesions are partly of membranous and partly of proliferative type. In Cases 150 and 156 the glomeruli resemble those shown in Figures 2 and 3 except that the basement membranes are much thicker. Case 154 differs from these only in the presence of many fresh epithelial crescents. Case 149 is a typical membranous type except for a few glomeruli of the proliferative form. Cases 148, 151, 152, 153 and 155 show only occasional

TABLE IX

Group IV. Subgroup B. Hydropic Type. Thick Basement Membranes, but many Glomeruli of Proliferative Type

Case No.	Autopsy No.	Age yrs.	Sex	Duration of symptoms	Albuminuria	Edema	Blood pressure	Urea nitrogen mg./100 cc.	Non-protein nitrogen mg./100 cc.	Phenolsulpho- phate %	Cholesterol	Plasma proteins gm. %	Weight of heart gm.	Weight of kidneys gm.	Hemoglobin %	Passive congest- ion of liver	Hyaline glomeruli	Tubular atrophy	Basement membrane	Cause of death	
148	35-1222	24	F	17 mos.	3	3	194/130 (2 days)	—	87.6 (2 days)	—	—	—	550	230	48	—	—	70	3	4	Hydro- thorax
149	37-271	27	F	4 mos.	3	2 1	136/80 (2 mos.)	—	12.6 (2 mos.) 46 (2 wks.)	70 (2 wks.)	—	4.2	310	475	42	0	5	0	4	Lobar pneu- monia	
150	29-1880	27	M	3 yrs.	3	3	160/100	9 (1 mo.)	36 (1 mo.)	65 (1 mo.)	—	a, 0.69 g, 3.26	—	—	74	0	10	1—	3	Peritoni- tis	
151	31-1672	37	M	16 mos.†	3	3	150/90	—	—	—	—	—	325	435	—	0	5	0	3	Hydrotho- rax and ascites	
152	20-43	38	M	5 yrs.	3	3	122/84 (1 mo.)	16.4 (1 mo.)	—	30 (1 mo.)	—	—	325	390	52	0	10	1—	2	Pneu- monia	
153	27-925	39	F	8 yrs.	—	2	—	—	—	—	—	—	264	267	20	0	0	0	2	Severe anemia	
154	17-230	45	M	1 yr.	2	4 1	140/70	24 (9 days)	—	30 (1 mo.)	—	—	400	360	—	0	0	2	2	Lobar pneumonia	
155	33-574	45	M	—	4	4 0	128/70 165/80 (3 wks.)	22 (3 wks.)	42 (3 wks.)	65 (3 wks.)	—	—	425	400	—	0	10	1—	3		
156	27-305	52	F	2 yrs.	†	1	—	—	—	—	—	—	315	350	80	0	10	1	3	Peritonitis from per- forated ulcer	

Under plasma proteins, a = albumin, g = globulin.

glomerular lobules of proliferative type, all the others being membranous.

The glomerular structure in Subgroup B is therefore a blending of proliferative and membranous lesions with a predominance of the latter. There is a much closer resemblance to lipid nephrosis than to azotemic glomerulonephritis.

Subgroup C (Table X): In the 25 cases in this group the clinical diagnosis was lipid nephrosis and the glomeruli show no evidence of proliferative glomerulitis. Six of the 25 cases show no visible alterations in the glomeruli. It is certain that these 6 cases correspond to what others have called pure nephrosis but none of them satisfies the arbitrary definition laid down by Leiter that there shall be no elevation of blood pressure or decrease in renal function. However, they satisfy the criteria suggested by Blackman in that there is no progressive hypertension or renal insufficiency. Clinically there are no distinctions between those with no changes in the basement membranes (Cases 157, 158, 159, 160, 162 and 163), those with patchy membrane thickening (Cases 161, 164, 165 and 173) and 2 of those with pronounced thickening of the basement membranes (Cases 166 and 167). The great majority of those with thick basement membranes show hypertension.

It is noteworthy that in the 11 children (Cases 157 to 167) the structural changes in the glomeruli were much less pronounced than in the adults. Thirteen of the 14 adults but only 2 of the 11 children showed a diffuse thickening of the basement membranes.

The plasma proteins were markedly reduced in 12 of the 13 cases in which they were determined. Cardiac failure plays no rôle in causing edema since passive congestion of the liver was present only in the 1 case with death from endocarditis.

There were 4 deaths from uremia and 17 from infectious processes. Twelve of the 17 infections were peritonitis, of which 5 were streptococcic, 2 pneumococcic and the others undetermined. An incomplete survey of the literature shows that peritonitis was assigned as the cause of death in 42 of 53 cases. Of these, 23 were pneumococcus infections, 10 streptococcic and 9 not specified.

The clinical course of the disease was usually characterized by alternating exacerbations and remissions, the symptoms being intense during the former and mild during the latter. In Case 159

TABLE X

Group IV. Subgroup C. Hydropic Type without Proliferative Glomerulitis. Thickened or Normal Capillary Basement Membranes

Case No.	Autopsy No.	Age	Sex	Duration of symptoms	Albuminuria	Edema	Blood pressure	Urea nitrogen mg./100 cc.	Non-protein nitrogen mg./100 cc.	Phenolsulpho- nephthalein %	Cholesterol	Plasma proteins gm. %	Weight of heart gm.	Weight of kidneys gm.	Hemoglobin %	Passive congestion of liver	Hyaline glomeruli %	Tubular atrophy	Basement membrane	Cause of death	Comment	
157	34-383	1.5 yrs.	M	6 wks.	4	3	114/70	—	—	—	—	—	—	300	—	—	0	0	0	0	Peritonitis	Onset with sore throat
158	31-886	3	F	7 mos.	4	4	90/50	31.5 (6 mos.) 10.7 (2 mos.)	—	33 (2 mos.)	240	—	65	—	190	50	0	0	0	0	Pneumococcic peritonitis	
159	35-461	4	M	16 mos.	4	4	110/68	21 (1 yr.) 58 (p. m.) 13 (1 yr.)	—	—	251	a, 1.07 g, 5.97 f, 0.8	75	250	82	0	0	0	0	0	Str. viridans peritonitis	Repeated attacks of peritonitis
160	35-242	5	F	15 mos.	1 4	3	94/68	71 (1 day)	—	—	244	a, 2.0 g, 2.2 f, 0.8	—	250	75	75	0	0	0	0	Peritonitis	Followed a cold
161	33-1579	5	M	2.5 yrs.	1 4	4	95/60 110/70	10.5 (1 mo.) 27.6 (3 wks.)	45 (1 mo.) 27.6 (3 wks.)	30 (1 mo.)	215	a, 1.65 g, 2.11	75	290	70	70	0	5	1p	3p	Streptococcic peritonitis	Followed pneumonia
162	26-625	6	F	6 mos.	3	4	—	27 (6 wks.) 19 (1 mo.) 11.7 (9 mos.) 29 (1 day)	—	15 (2 wks.)	—	—	60	275	68	—	5	1p	0	0	Peritonitis	
163	33-1472	7	M	13 mos.	4	3	96/66	45.8 (10 mos.) 75.8 (1 day)	—	40 (9 mos.) 74 (1 mo.)	—	a, 1.8 g, 2.8 f, 0.4	290	267	—	0	0	0	0	0	Peritonitis	
164	36-472	7	M	5 mos.	4	2	—	22 (3 wks.) 48 (2 yrs.) 99.8 (2 wks.)	—	—	452 1025	a, 1.33 g, 3.56 a, 0.6 g, 3.16	175	310	—	0	0	0	1p	1p	Erysipelas	
165	32-1297	9	F	2 yrs.	0 4	4 0	108/88	15.4 (2 yrs.)	—	—	—	—	180	455	56	0	0	0	0	1p	Streptococcic peritonitis	
166	30-159	12	F	7 mos.	3	4	110/80	10.3 (7 mos.) 61.2 (1 day)	—	67 (7 mos.) 42 (1 mo.)	—	—	130	460	45	0	0	2	3	3	Streptococcic peritonitis	Hematuria crescents
167	0-38-820	13	F	5 mos.	2 4	4	116/82	—	—	—	609	2.71 4.2	170	425	—	—	0	0	2	2	Pneumococcic peritonitis	

168	30-1856	20	F	22 mos.	1	2	140/70 (22 mos.) 222/134 (4 mos.)	77 (22 mos.) 102 (1 day)	—	54 (22 mos.)	—	—	484	254	45	0	10	2	3	Uremia, pneumo- coccic bacterie- mia	Retinitis	Followed sore throat
169	30-768	21	M	—	3	4	180/100 (1 wk.)	53.9 (9 days)	—	—	—	—	387	549	—	0	0	2	2	Peritonitis		
170	32-1080	31	M	6 yrs.	1	2	158/96 (6 mos.)	—	41.4 (6 mos.) 166.5 5	65 (6 mos.)	a, 0.9 g, 2.6	525	400	69	0	10	3	3	3	Uremia		
171	34-1536	32	F	2 yrs.	4	3	130/98 (2 yrs.)	31.2 (2 yrs.) 120.4	230	50 (2 yrs.)	a, 1.49 g, 1.09 f, 0.66	440	170	44	0	70	3	3	3	Uremia		
172	28-183	34	F	7 mos.	3	3	200/140 (1 mo.)	47 (1 wk.)	—	0 (1 wk.)	—	300 (one)	300	48	—	—	0	1	2	Broncho- pneumonia		
173	35-654	36	M	5 wks.	4	3	108/74	—	38 (2 wks.)	—	a, 1.0 g, 0.9 f, 0.7	280	365	73	—	—	0	0	ip	Accident		
174	37-2146	36	M	1 yr.	3	3	130/90 (2 mos.)	28.7 (2 mos.)	—	15	a, 2.97 g, 1.83 f, 0.33	340	420	70	—	—	0	0	1	Uremia		
175	25-636	37	M	5 yrs. +	2	2	150/120 170/120 (2 mos.)	31 (2 mos.) 159 (5 days)	—	—	—	450	375	—	1	50	3p	3	3	Uremia		
176	35-961	40	M	9 mos.	3	4	130/90 (8 mos.)	18 (8 mos.)	36 (8 mos.)	65 (8 mos.)	a, 1.76 g, 1.68 f, 0.46	325	485	—	1	0	0	2	2	Bacterial endocarditis		Followed sore throat 5 yrs. as pure type
177	32-1127	43	M	7 yrs.	4	4	Normal to 250/?	Normal to very high	—	—	—	500	225	—	0	90	4	3	3	Uremia		
178	31-1408	48	M	4 mos.	4	2	148/102 (4 mos.)	—	77 (4 mos.) 95 (1 day)	55 (4 mos.)	—	Normal	305	—	—	0	10	2	3	Inanition		
179	17-145	54	M	1 yr.	2	4	162/104 (1 mo.)	13 (10 mos.) 23 (10 days)	—	72 (10 mos.)	—	320	450	—	—	10	ip	2	2	Purulent bronchitis		
180	35-114	58	M	3.5 mos.	3	3	140/90	15.9 (2 wks.)	—	44 (2 wks.)	a, 1.91 g, 2.62	250	270	56	0	0	0	2	2	Streptococ- cic perito- nitis		
181	26-827	62	F	15 yrs.	3	3	134/82 (2 days)	25 (1 day)	—	—	—	330	260	40	0	0	0	4	4	Severe anemia		

Under plasma proteins, a = albumin, b = globulin, f = fibrinogen.

there were repeated attacks of peritonitis. The exacerbations often followed an upper respiratory infection. The duration of symptoms was only 5 and 6 weeks in Cases 173 and 157, and these might appropriately be classified as acute, but the duration in the other cases warrants the diagnosis of subacute or chronic.

Cardiac hypertrophy was found in those who died of uremia.

The Structural Alterations in the Kidneys: The convoluted tubules often contain numerous droplets of lipoid, but they never show primary degeneration or necrosis. Atrophy of the tubule occurs only when the capillaries of its associated glomerulus become obstructed; there is no primary tubular atrophy. Lipoid nephrosis is not a primary tubular disease.

In 6 instances there were no visible changes in the capillary basement membranes. Cases of this type have been reported by several writers and have given rise to the widespread belief that in lipoid nephrosis the glomeruli are normal. It appears from our study (Table X) that in young children the basement membranes either show no thickening at all (Fig. 9) or only focal areas of thickening (1p, in Table X), while in older children and adults thickening of the basement membranes is nearly always pronounced. The absence of thickening is not due entirely to a short duration of the disease, since in 3 cases without thickening the duration was 13 mos., 15 mos. and 16 mos. respectively. It seems highly improbable that these cases with normal appearing capillaries represent a different disease from those with thick membranes, since the clinical features of the two groups are almost identical and there are numerous gradations from normal membranes to those that are very thick. In view of the remarkable permeability of the capillaries to the plasma proteins one must believe that the capillary walls, *i.e.* the basement membranes, are injured even though they show no structural changes. The capacity of the membrane to thicken in response to the injury is in some way related to age.

In Cases 164, 165 and 173 there were individual glomerular lobules here and there with thick basement membranes, and in Case 161 there were a few glomeruli with marked diffuse thickening of the basement membranes (Fig. 10). Blackman noted a few hyaline glomeruli in several of his cases, but explained them as a result of focal glomerulonephritis. However, these individual glo-

meruli show the same thickening of the basement membranes as is found in the diffuse form.

Tubular atrophy occurs when the thickened basement membranes have produced marked narrowing or closure of the glomerular capillaries. Complete closure of the capillaries results in hyalinization of the glomerulus and extreme atrophy of its tubule. When a large proportion of the glomeruli have become hyaline the kidneys may shrink in size and uremia develops (Cases 171, 175 and 177) (Fig. 11). It is to be noted that the contracted kidneys of lipid nephrosis are due to primary glomerular disease with secondary tubular atrophy and not to primary tubular degeneration as Th. Fahr maintained. When a small proportion of the glomeruli are hyaline (Cases 178, 179) a patchy type of atrophy develops. A diffuse tubular atrophy of moderate degree develops when all the glomerular capillaries are narrowed but not completely closed (Cases 166, 168, 169, 170, 171) (Fig. 12). Uremia may develop in this way before any large proportion of the glomeruli have become hyaline (Case 170).

THE RELATION OF HYDROPIG GLOMERULONEPHRITIS (LIPOID NEPHROSIS) TO THE AZOTEMIC TYPE

In the foregoing discussion we have presented clinical and anatomical evidence that lipid nephrosis is a glomerular and not a tubular disease, but since this view is not widely accepted at present a brief historical résumé of the subject may be helpful.

Prior to 1914 lipid nephrosis was known as parenchymatous nephritis. It was well known that edema and albuminuria were outstanding features, that uremia seldom developed, and that the kidneys were usually large. At that time azotemic glomerulonephritis and the hypertensive kidney were regarded as disease of the interstitial tissue, "interstitial nephritis" in contrast with the "parenchymatous" type which was vaguely considered tubular disease.

The identification of glomerulonephritis by Langhans and Löhlein and of vascular disease by Ziegler initiated the modern period of renal investigation. The popular monograph by Volhard and Fahr in 1914⁷ created widespread interest in nephritis, but their effort to establish "nephrosis" as an entity has retarded progress. These writers classed as nephroses all renal diseases which

they considered degenerative in nature, such as the effects of chemical and bacterial poisons, amyloid disease, eclampsia and genuine or lipid nephrosis. These diseases have little in common either clinically or pathologically and nothing is to be gained by placing them in one group. In recent years "nephrosis" has been restricted by most writers to lipid nephrosis.

Volhard and Fahr distinguished two forms of lipid nephrosis — pure nephrosis and nephrosis with a nephritic component. They believed that nephrosis and nephritis are distinct diseases, the former being degenerative in character, the latter inflammatory. When a patient with the nephrotic syndrome (albuminuria, edema, and so on) developed hypertension or uremia, they believed that nephritis had been superimposed on nephrosis. They stated that pure nephrosis shows only tubular degeneration and that nephritis shows inflammation in the glomeruli. These ideas are still widely supported by clinicians and pathologists.

Another theory of lipid nephrosis that has many adherents is that it is a general metabolic disorder with secondary renal changes. This view was supported by Epstein⁸ who wrote of "albuminuric diabetes." Diebold,⁹ Wolbach and Blackfan¹⁰ and others do not believe that the renal lesions are responsible for the symptoms.

It is customary to describe two forms of lipid nephrosis — the pure type and the mixed type. There are many who believe that these forms are distinct and that the mixed type is a form of glomerulonephritis; others regard the mixed type as a mixture of nephrosis and nephritis, and a few believe that nephrosis is merely a variety of glomerulonephritis.

(A) *Pure Lipoid Nephrosis*: This disease is characterized clinically by the presence of marked edema, albuminuria, hypercholesterolemia and low plasma proteins, and by the absence of hypertension, hematuria and renal insufficiency. Pathologically the usual descriptions emphasize the presence of abundant lipid in the renal tubules and the absence of glomerular disease.

There is disagreement in the literature as to the clinical limitations of the disease. The most rigid definition is given by Leiter¹¹ who excludes from this group every case in which there is hematuria, any elevation of blood pressure or any renal insufficiency. Volhard¹² apparently holds a somewhat similar view. But the

majority of writers adopt a more elastic definition. Gainsborough¹³ found hematuria at the onset of the illness in 6 of 10 cases, and several other writers mention hematuria in an occasional case. One of our cases, Case 168, showed hematuria. Blackman¹⁴ does not exclude cases that show transitory hypertension or increases of non-protein nitrogen, but he would reject any case with a constant or progressive increase of non-protein nitrogen or of blood pressure. He would also reject those with gross hematuria. Many of our cases in Table IX would be admitted by Blackman's definition but would be excluded by Leiter's. When the patient is studied thoroughly with repeated and varied functional tests some degree of nitrogen retention will usually be found at times. The blood urea may be somewhat elevated early in the disease but normal later on (Cases 158 and 162). These variations are probably due to extrarenal influences.

With regard to the pathological changes in the kidneys the great majority of authors find the glomeruli normal. Blackman found a few hyaline glomeruli with atrophic tubules in several of his cases. There were focal thickenings of the basement membranes in 4 of our cases (Cases 161, 164, 165 and 173). In Case 166 there was a marked diffuse thickening of all the basement membranes with a beginning diffuse tubular atrophy, but some would reject this case because of the high urea nitrogen on the day of death.

(B) *The Mixed Type of Lipoid Nephrosis*: As noted above, this disease has all the positive features of the pure type but has in addition either azotemia or hypertension, or both conditions. In the literature there are three theories in regard to the nature of this disease: (1) that it is a form of glomerulonephritis entirely distinct from pure lipoid nephrosis; (2) that it is a nephrosis with a superimposed nephritis, or *vice versa*; and (3) that it is a variety of glomerulonephritis closely related to pure lipoid nephrosis and that its special symptomatology is due to the anatomical nature of its glomerular lesions.

Many writers discuss the "nephrotic syndrome" or nephrosis including cases of both the pure and the mixed types, and do not concern themselves with the anatomical nature of the lesion.

(1) The view that pure nephrosis is different from the mixed type is supported by many writers on the basis of their experience. They have observed cases of nephrosis without persistent hyper-

tension or azotemia where the individual either recovered entirely or died of an infection, usually peritonitis. At postmortem no proliferative glomerulitis was found. But these writers offer no explanation for the cases that exhibit the symptoms of pure lipoid nephrosis for some years and then develop hypertension and uremia. Several such cases are now on record. Volhard¹² mentioned a case of nephrosis in a boy 7 years of age, who developed clinical evidence of a nephrotic contracted kidney. Débre and Marie¹⁵ reported the case of a child 5 years of age who died in uremic coma after a period of 3 years of pure lipoid nephrosis. Gainsborough¹³ reported that one of his patients had nephrosis for 8 years and developed slight hypertension and nitrogen retention during the last few months of life. A remarkable case was reported by George Fahr¹⁶ (Case 177). The patient, a physician, was 36 years of age at the onset of his illness. For 5 years he had a typical picture of pure lipoid nephrosis and was studied carefully in several prominent clinics. During the last 2 years of his life he gradually developed hypertension and azotemia, and died with a very high blood pressure and a marked elevation of blood urea.

It is, therefore, well established that pure lipoid nephrosis may pass gradually into the mixed type, developing hypertension and uremia. Those who insist on the separate identity of the pure and mixed types can only suppose that nephritis has been superimposed on the nephrosis, but as we shall show presently no new disease has been introduced but the glomerular capillaries have been progressively narrowed and closed by thickening of the basement membranes.

(2) The second view is that the mixed type is a mixture of two diseases, *i.e.* nephrosis and nephritis. This view was first promulgated by Volhard and Fahr who used the expression "nephritis with a nephrotic Einschlag." It is not entirely clear what these authors meant by a "nephrotic Einschlag." Volhard explained some years later that he meant nephritis with a tendency to edema, but Fahr has stated definitely that nephrosis is something quite distinct from nephritis.

The erroneous view has been widely accepted that albuminuria and edema indicate nephrosis and that hypertension and azotemia mean nephritis. But albuminuria and edema occur also in nephritis although usually in lesser intensity, and consistency re-

quires the advocates of this theory to admit some nephrotic component in most cases of nephritis. There is no feature of nephrosis that does not occur also in some cases of nephritis. Furthermore it has been clearly established that albuminuria and edema are due to glomerular and not to tubular disease.

(3) The third theory postulates that the mixed type of lipid nephrosis is a variety of glomerulonephritis closely related to pure lipid nephrosis, and that its symptoms may be explained by the nature of the glomerular lesions. We have advocated this interpretation for several years.

Résumé of the Pathology of Hydropic Glomerulonephritis (Lipoid Nephrosis): The clinical syndrome, commonly called lipid nephrosis, is not associated with a uniform type of glomerular lesion. In 6 of our 40 cases there was a proliferative glomerulonephritis but most of the glomerular capillaries were patent, allowing the escape of serum proteins into the urine and thus favoring the development of edema.

In 6 young children there were no visible changes in the glomeruli. In 3 children and 1 adult individual glomeruli or individual glomerular lobules showed thickening of the capillary basement membranes. In the other 24 cases there was a definite diffuse thickening of the membranes. Two children, aged 12 years and 13 years respectively, showed this membranous change as strikingly as the adults. In nine adults (Table IX) some glomerular lobules showed proliferative changes. In 3 of the 4 cases with death from uremia a large proportion of the glomeruli were hyaline.

Apart from the 6 cases of proliferative glomerulonephritis mentioned above, lipid nephrosis was associated with a membranous type of glomerulitis when any lesions were visible. There were no clinical distinctions between the 2 cases in children (Cases 166 and 167) with thick membranes and those with normal membranes. In those with diffuse thickening of the basement membranes hypertension was present in 14 and absent in 8 cases.

A moderate tubular atrophy develops when the glomerular capillaries become so narrow that they transmit a decreased amount of blood (Cases 166, 169 and 178), and the tubules disappear almost completely after the glomeruli become hyaline. The glomeruli are obliterated by progressive thickening of the

basement membranes. In the cases with death from uremia tubular atrophy is very pronounced. The atrophy of the tubules is not due to primary tubular disease but is secondary to the closure of the glomeruli. The "nephrotic contracted kidney" results from membranous glomerulonephritis.

DISCUSSION

In the various forms of glomerulonephritis the symptoms and the clinical course are closely dependent upon the character and the extent of the glomerular lesions. If the initial acute attack results in widespread severe capillary obstruction renal insufficiency soon develops. Those cases that terminate in uremia within a few months are called acute, while those that survive from 4 or 5 months to 1 year are usually called subacute. When the initial glomerular injury is less intense so that a majority of the capillaries remain more or less permeable a chronic nephritis develops. Complete anatomical recovery evidently takes place after mild acute glomerulonephritis. The initial lesions are less severe and extensive in the cases that become chronic than in those that follow a subacute course.

The initial glomerular lesion consists of an increase of endothelial cells and splitting and fragmentation of the central capillary basement membranes in the interior of the lobules (Fig. 4). If the capillaries become completely occluded the glomerulus becomes hyaline; if partially occluded a peripheral circulation develops in the lobule and the hyaline fibers, derivatives of the central membranes, become fused into a hyaline mass at the center of the lobule (Figs. 3 and 7). The glomeruli shown in Figures 2, 3 and 7 represent the usual structure of functioning glomeruli in chronic azotemic glomerulonephritis. Their structure is definitely altered but evidently glomerular filtration is not notably reduced since the associated tubules are not atrophic.

In latent chronic glomerulonephritis nearly all of the glomeruli have a structure similar to that shown in Figures 2 and 3. If the lesion does not progress beyond this stage renal function remains adequate. In advanced chronic glomerulonephritis many of the persistent glomeruli have a structure similar to those of the latent stage. The chief difference between the latent and advanced stages is that in the latter most of the glomeruli are either hyaline

or markedly obstructed. Azotemic glomerulonephritis is characterized by obstruction of the glomerular capillaries.

In hydropic glomerulonephritis the capillary walls are injured but the lumens remain open. This type of lesion seldom occurs in proliferative glomerulitis but it is characteristic of membranous glomerulitis. It is not known whether or not membranous glomerulitis has a different etiology from the proliferative form; we know only that in the proliferative type the capillaries become obstructed, while in the membranous form the capillary walls are injured and become permeable to the plasma proteins. The marked permeability of the capillaries to proteins causes edema, which is the outstanding feature of the disease. Hypertension does not develop until the thickened membranes have produced a definite narrowing of the capillary lumens. Extreme thickening of the membranes may result in extensive hyalinization of the glomeruli and renal insufficiency. For some unknown reason hydropic glomerulonephritis in young children seldom shows extensive thickening of the basement membranes.

Azotemic nephritis is due to capillary obstruction and hydropic nephritis results from increased permeability of the capillaries to proteins. Azotemia develops regularly in proliferative glomerulitis but infrequently in the hydropic form. Hydropic glomerulonephritis is usually due to membranous glomerulitis, occasionally to the proliferative form.

SUMMARY

Of 181 cases of glomerulonephritis, 16 were classified as subacute, 8 as latent chronic, 117 as chronic azotemic and 40 as chronic hydropic.

In subacute glomerulonephritis the kidneys are not contracted. There is widespread severe glomerular obstruction with well advanced uniform tubular atrophy. There are few hyaline glomeruli.

There are 125 cases of chronic azotemic glomerulonephritis. Eight cases of latent chronic glomerulonephritis are described in which death was due to an intercurrent disease. Only 1 such case has been reported previously. There are only a few hyaline glomeruli and there is little or no tubular atrophy. The glomeruli are all damaged to some degree, their lobules showing hyaline central portions and peripheral capillaries.

Thirty cases of chronic azotemic glomerulonephritis are reported in which there is a history of an initial acute attack. The total duration varied from 1.5 years to 26 years, with an average duration of 10 years. In 15 of the 30 cases the acute attack was followed by a latent chronic stage varying from 1 year to 24.5 years in length; in the remaining 15 cases the acute stage passed directly into active chronic nephritis.

In 30 per cent of the cases the systolic blood pressure was 200 mm. Hg. or higher.

There is some degree of chronic passive congestion of the liver in nearly one-half of the cases, indicating some degree of heart failure, but there is no evidence that heart failure is ever more than a contributing cause of death since all of the patients had uremia. Heart failure may occasionally be a contributory cause of edema. Only one patient had a history of apoplexy and only one had an attack of coronary sclerosis.

Retinitis was found in 35 of 46 cases in which the eyegrounds were examined. There is a definite relation between high blood pressure and retinitis.

There is no relation between the weight of the kidneys and the duration of the disease or the height of the recorded blood pressure. The kidneys are occasionally of normal size or even enlarged in the terminal stages. Large kidneys contain a high proportion of injured glomeruli with moderately atrophic tubules, while small kidneys consist largely of hyaline glomeruli with extremely atrophic tubules.

Forty cases of hydropic glomerulonephritis (lipoid nephrosis) are reported. In 6 of these the glomerular structure was that of chronic proliferative glomerulonephritis, and in 9 others there was a mixture of proliferative and membranous glomerular lesions with the latter in great preponderance. In the remaining 25 cases there were no proliferative lesions.

In 6 cases in young children there were no visible changes in the glomerular capillaries, and in 3 other children there were only focal membranous lesions.

With one exception diffuse thickening of the basement membranes was present in all persons over 12 years of age.

In 4 cases membranous glomerulitis produced such an extensive narrowing of the glomerular capillaries that uremia developed.

When a patient with pure lipoid nephrosis develops hypertension and uremia no new disease is superimposed — there is merely progressive thickening of the basement membranes.

Nephrosis is a form of glomerulonephritis in which the glomerular capillaries remain open and allow the blood proteins to escape into the urine. In proliferative glomerulonephritis the capillary lesions are nearly always of obstructive type.

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DESCRIPTION OF PLATES

PLATE 142

- FIG. 1. Case 2. Subacute glomerulonephritis. Note obstruction of glomerular capillaries and moderate tubular atrophy. $\times 200$.
- FIG. 2. Case 18. Latent chronic nephritis. Note absence of tubular atrophy. The glomerular lobules show solid central portions with peripheral capillaries. $\times 200$.
- FIG. 3. Case 18. Latent chronic nephritis. Detailed structure of glomerular lobule. Note hyaline central portions of lobule and peripheral capillaries. $\times 850$.
- FIG. 4. Glomerular lobule from mild acute glomerulonephritis. Note fragmentation of the central capillary basement membranes and partial permeability of capillaries. This is probably the type of lesion that becomes chronic. $\times 850$.

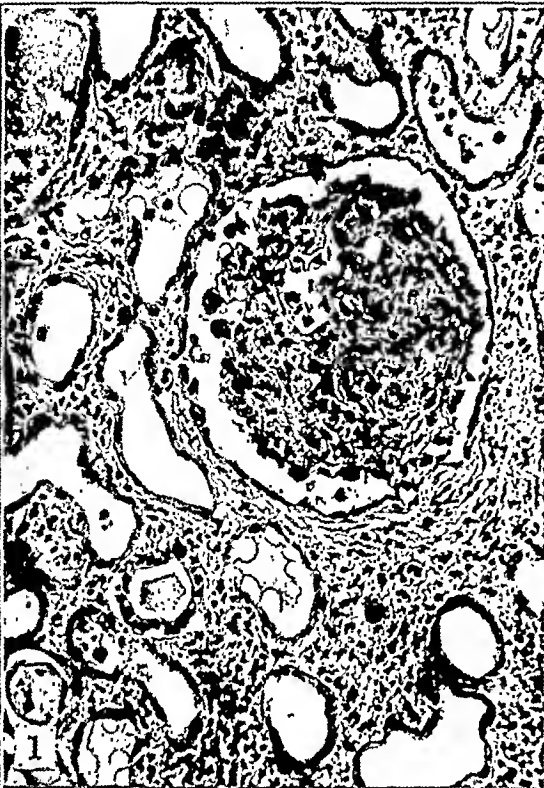


PLATE 143

- FIG. 5. Case 93. Chronic azotemic glomerulonephritis with contracted kidneys. Histological type *a*. Note large proportion of hyaline glomeruli. $\times 80$.
- FIG. 6. Case 25. Chronic azotemic glomerulonephritis with uremia. Weight of kidneys 460 gm. Duration 4 years. Histological type *b*₁. Partially obstructed glomeruli with well advanced tubular atrophy. $\times 200$.
- FIG. 7. Case 57. Chronic azotemic glomerulonephritis with uremia. Histological type *b*₁. Lobule of a glomerulus showing central hyaline mass and peripheral capillaries. $\times 850$.
- FIG. 8. Case 142. Group IV. A. Chronic hydropic glomerulonephritis with the microscopic structure of proliferative glomerulonephritis. $\times 200$.



Bell

Subacute and Chronic Glomerulonephritis

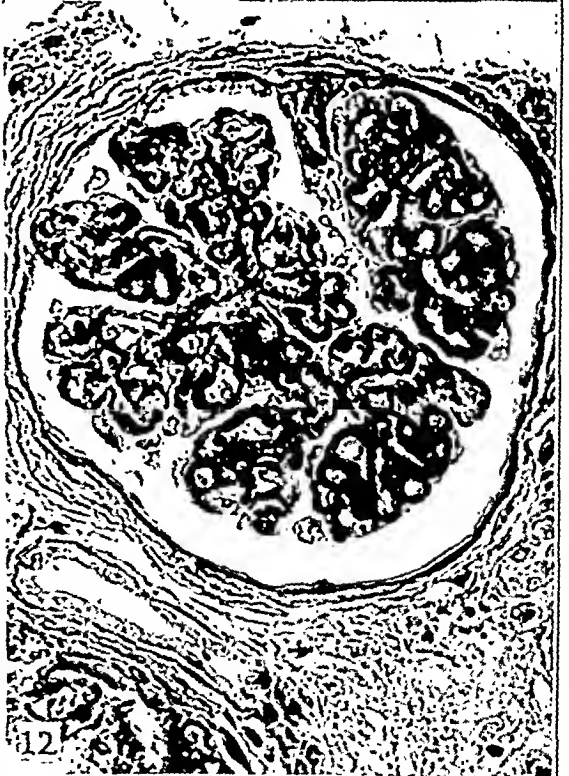
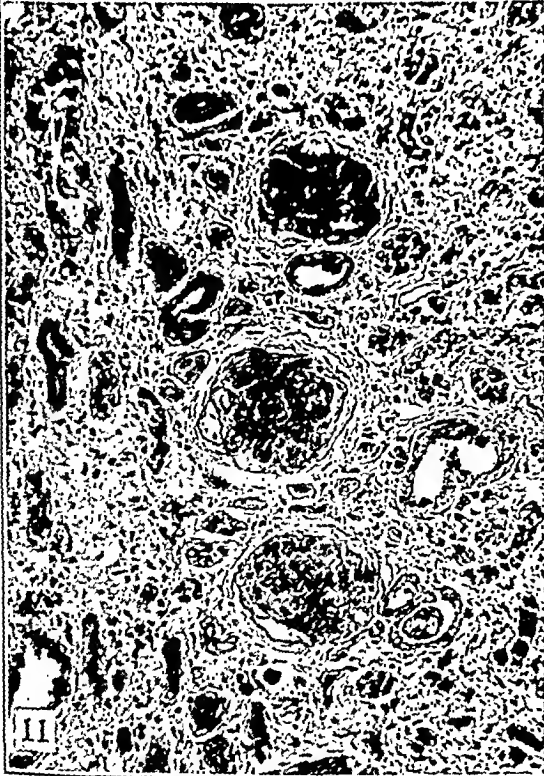
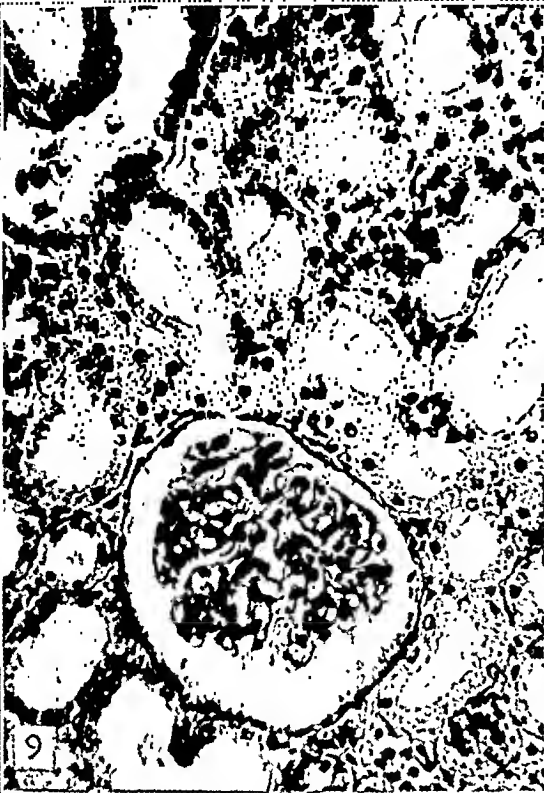
PLATE 144

FIG. 9. Case 164. Hydropic glomerulonephritis in a child 7 years of age. There are no structural changes in the glomeruli. $\times 200$.

FIG. 10. Case 161. Hydropic glomerulonephritis in a child 5 years of age. A majority of the glomeruli show no changes. The illustration shows an individual glomerulus with diffuse thickening of the basement membranes. Mallory-Heidenhain stain. $\times 400$.

FIG. 11. Case 171. Chronic hydropic glomerulonephritis with contracted kidneys and uremia. Note small hyaline glomeruli and atrophic tubules. $\times 150$.

FIG. 12. Case 168. Chronic hydropic glomerulonephritis. A glomerulus stained by the Mallory-Heidenhain method. Ninety per cent of the glomeruli were of this structure. Note the thick basement membranes. $\times 400$.



ACUTE HEMATOGENOUS INTERSTITIAL NEPHRITIS *

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INTRODUCTION

In acute hematogenous interstitial nephritis the kidney is usually enlarged and soft, the parenchyma moist, and the glistening cut surface reveals a grayish streaking of the thickened cortex. Histological examination shows that the tubules are separated by interstitial accumulations of fluid with varying amounts of cellular exudate. Most pathologists are familiar with this condition but little attention hitherto has been paid to it. The lesion is encountered most frequently incidentally at autopsy and, since no disturbance of renal function is generally suspected, the findings are considered to be irrelevant. Occasionally, however, cases are observed where failure of renal function, progressive oliguria and uremia are outstanding features and interstitial nephritis is found to be the only, or at least the most prominent, morphological finding. This has prompted Fahr and others to recognize interstitial nephritis as a disease entity. Fahr states that this type of "inflammatory swelling of the kidney is of importance because it may result in oliguria and possibly anuria with subsequent uremia."

Although it is true that interstitial nephritis may occasionally gain considerable significance, we know as yet little about its pathogenesis, its correlation to functional disturbances and simultaneous changes in any other organs. Interstitial nephritis is not much more than a mere morphological concept. Our knowledge of the multitude of conditions anteceding or causing this lesion in the kidney is incomplete. The denominator common to all has not yet been found. We are still unable to decide to what extent the renal lesion participates in the production of coinciding anuric uremia. Although extrarenal factors seem to play a dominating rôle, it has been shown that the kidney function is actually disturbed.

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The following study was prompted by the observation of several very striking cases of interstitial nephritis with anuria and uremia. These and a number of "silent" cases of interstitial nephritis, found incidentally at autopsy, will be reported briefly, and their significance in regard to the above mentioned problems will be discussed.

CASE REPORTS

CASE I. Mrs. M. A. (A-2306), a pregnant white female, 33 years of age, was admitted to the Memorial Hospital with the chief complaint of discharge from the vagina and persistent vomiting. Five days previously the patient had inserted a rubber catheter into the cervix. The pregnancy had progressed

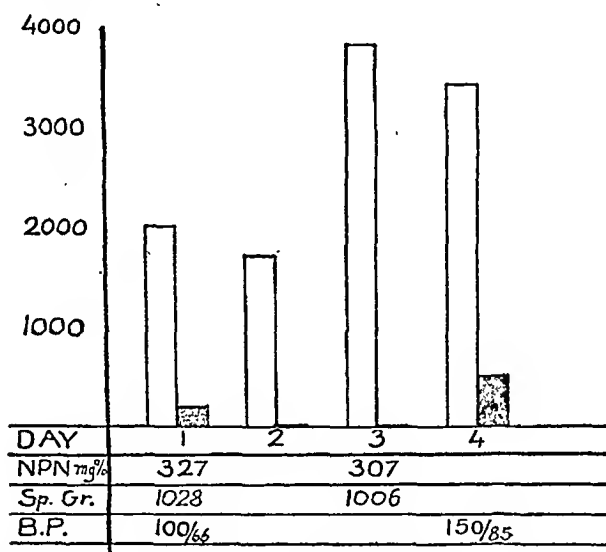


Chart 1. Case 1, A-2306. White column = fluid intake.
Black column = urine output.

uneventfully, but 2 days before admission to the hospital she began to have abdominal cramps, following which a large blood clot was passed. Since then she had had a continuous, foul smelling, blood-tinged vaginal discharge. She had one chill and the night before admission began vomiting everything, including fluids. There had been no bleeding for the past 8 days. The past history was insignificant.

Physical Examination: A soft systolic murmur was heard at the apex of the heart. The blood pressure was 100/56 mm. Hg. The uterus was not palpable and there was no tenderness or rigidity. There was a foul smelling, grayish white vaginal discharge but no bleeding. The physical examination was otherwise negative.

Course of Illness: The patient continued to vomit, the vomitus containing small amounts of bright red blood. The abdomen became slightly distended and tender. The temperature ranged between 98° and 100° F., and death occurred in uremia.

Autopsy Diagnoses: Infected postabortive endometrium; acute gangrenous appendicitis; fibrinous pericarditis; pulmonary edema and atelectasis; bilateral hydrothorax; ascites; and old, healed endocarditis of mitral valve.

Kidneys: Weight 250 gm. each. In gross the characteristic picture of interstitial nephritis, except for the presence of punctate hemorrhages on the surface, was seen. The differential diagnosis between acute early glomerulonephritis and interstitial nephritis could not be made in gross.

Histological Examination: There is severe edema with separation of the tubules. The cellular infiltration, which is slightly more marked in the medulla than in the cortex, consists mainly of small round cells and polymorphonuclear leukocytes. The pelvises are free from infiltration. The tubules are markedly dilated. Degenerative changes of epithelial cells is negligible. Hemoglobin and hematin casts are present. The glomeruli show questionable intercapillary edema but no inflammation.

Summary: The case is that of an abortion with infected placental residue and acute gangrenous appendicitis. There was no septicemia. Severe oliguria and temporary anuria developed, for which circulatory failure cannot be considered the cause (terminal blood pressure 150/85 mm. Hg.). The specific gravity of the urine was low toward the end, the blood pressure raised, the non-protein nitrogen high, and death occurred in uremia with uremic pericarditis.

The interstitial nephritis in this case is of the serous type.

CASE 2. W. S. P. (A-2156), a white female, 33 years of age, was admitted to the Memorial Hospital because of jaundice, abdominal pain, chills and fever. After missing a menstrual period the patient considered herself pregnant and took one drachm of ergot 3 times daily for 2 weeks. Two days before admission she took a lysol douche. A short time following this she began to have vague generalized abdominal pains which became more severe, and abdominal tenderness became marked. Jaundice developed 1 day before admission and was increased in intensity. She had fever, chills, headache, pain and edema of the ankles, bleeding from the vagina, and syncope with unconsciousness occurred the night before admission.

Physical Examination: The patient was a well developed and well nourished white obese female, markedly jaundiced. The temperature was 103.8° F., the pulse 114, the respiration 24, and the blood pressure 70/10 mm. Hg. The heart sounds were faint and distant. There were no signs of pulmonary disease. The abdomen showed no tenderness. A slight edema of the lower extremities was present.

Course of Illness: The treatment consisted essentially of supportive measures. Parenteral fluids and transfusions of blood (approximately 400 cc.) were given on the 1st, 2nd, 3rd, 4th, 7th, 8th, 11th, 12th and 13th days. The temperature ranged between 99° and 103° F. The patient's condition became progressively worse and twitching of the muscles of the face developed 2 days ante mortem with convulsions the night before death. She died in uremia 16 days after admission.

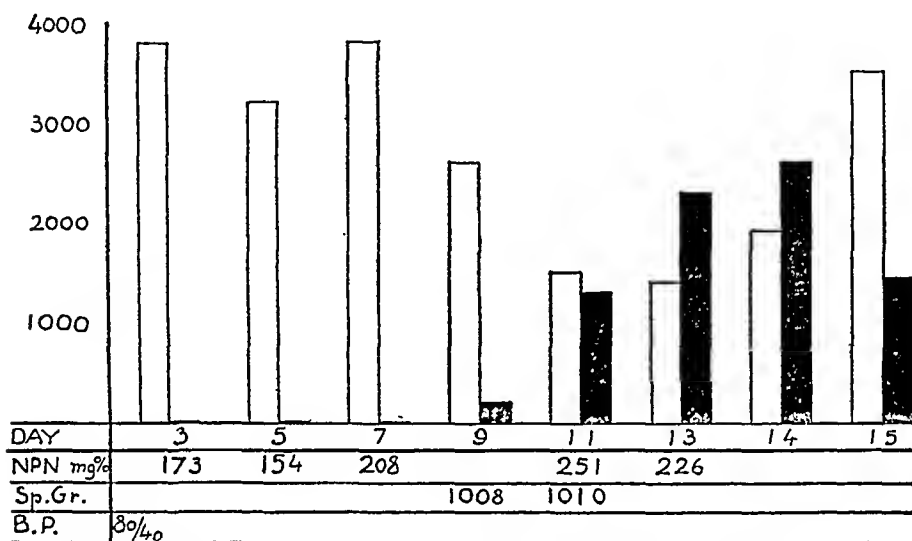


Chart 2. Case 2, A-2156. White column = fluid intake.
Black column = urine output.

Autopsy Diagnoses: Severe chemical burns of the vagina with excoriation of the skin and external genitalia; marked edema of the urinary bladder; and confluent bronchopneumonia of the right upper lobe.

Kidneys: Weight 350 gm. each. The grossly typical picture of interstitial nephritis was seen.

Histological Examination: There is severe intertubular edema. The cellular infiltration is evenly distributed between the medulla and the cortex and consists of small round cells, plasma cells, eosinophils and a few polymorphonuclear leukocytes. The tubules show severe dilatation with marked degenerative changes including occasional epithelial necrosis. Hemoglobin and hematin casts are present in almost all collecting tubules of the medulla. Most of the tubules are seemingly blocked by hematin casts. The glomeruli are essentially negative.

Liver: There is moderate degeneration of the liver cells, char-

acterized by basophilic granulation and a great irregularity of the nuclei with dissociation of liver cells in the center of the lobules.

Summary: The case is that of a chemical burn of the vagina and skin of the external genitalia followed by severe toxemia and jaundice. There was no septicemia. The condition was associated with temporarily complete anuria, the specific gravity of the urine was low, the non-protein nitrogen was raised, and death occurred in uremia in spite of reestablished urinary excretion.

The interstitial nephritis at the time of death was most severe and of the serous type.

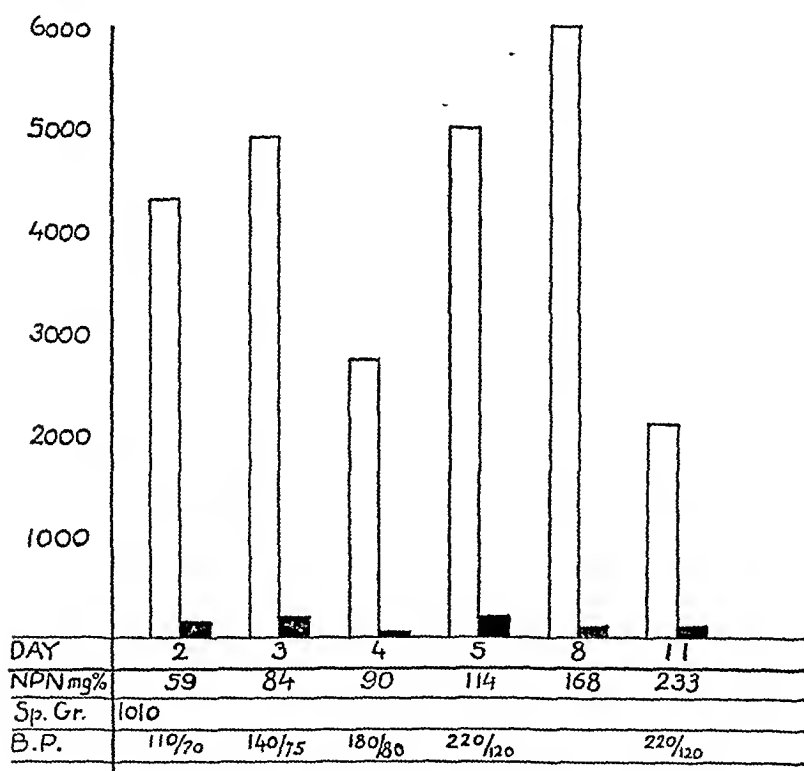


Chart 3. Case 3, A-2003. White column = fluid intake.
Black column = urine output.

CASE 3. This patient (A-2003), a 43 year old white male, was admitted to the Memorial Hospital on Aug. 25, 1936, complaining of pain over the left kidney of 3 weeks duration. There was a history of some burning upon urination and nocturia. The diagnosis at that time was ureteral calculus with acute pyelitis and pyelonephritis. On Sept. 2, 1936, the patient was operated upon and a ureteral calculus was removed from the left ureter. The blood pressure was 160/110 mm. Hg., the non-protein nitrogen 57, and the urea 38 mg. per cent. Three weeks later the patient was readmitted to the hospital and an intracapsular nephrectomy (left side) was performed Oct. 4, 1936.

The patient was readmitted again on Jan. 1, 1937, complaining of abdominal pain on the right side. There was no cardiac hypertrophy. The blood pressure was 130/90 mm. Hg. Two days after admission he was believed to have an obstruction of the right ureter and a right nephrotomy was done. The operative wound continued to drain for 10 days and the patient finally succumbed in uremia. Two blood transfusions were given (500 cc. each) on the 2nd and 7th days.

Autopsy Diagnoses: Absence of left kidney, postoperative; nephrotomy, right; lobular pneumonia in right upper lobe with hemorrhage and abscess formation; fibrinous pericarditis; slight hydrothorax and ascites; and old healed endocarditis of aortic cusps.

Kidneys: The weight was not noted. The organ was greatly enlarged, very moist and in gross typical of interstitial nephritis.

Histological Examination: There is severe edema; the cellular infiltration, much more marked in the cortex than in the medulla, consists of monocytes, lymphocytes, eosinophils and a few polymorphonuclear leukocytes. The pelvis does not show any inflammatory infiltration. The tubules are markedly dilated and show a diffuse and most severe vacuolic disorder of the cytoplasm. There is also rather marked degeneration, occasionally of the necrotizing type. Hemoglobin casts are absent.

Liver: There is extensive vacuolization of the liver cells of the central portion of the lobules, resembling marked glycogen storage.

Summary: The case is that of an interstitial nephritis following pyelonephritis with nephrectomy of the opposite kidney. Absence of pelvic inflammation and the predominance of cortical infiltration indicate that the interstitial nephritis is of hematogenous origin rather than ascending. There was complete urinary suppression with a steadily rising non-protein nitrogen. Circulatory failure can be excluded as the cause of oliguria since the blood pressure rose toward the end to 220/120 mm. Hg.

The interstitial nephritis is of a mixed serous and cellular type.

CASE 4. E. C. (A-2073), a negro, 45 years of age, was admitted to the St. Philip Hospital because of severe and extensive burns over the left lower extremities and left hand. A spray of 4 per cent solution of tannic acid was applied with adequate sedation for relief of pain.

Course of Illness: The temperature rose to 103° F. the day after admission, stayed high and rose terminally to 106° F. The patient developed a small abscess at the left elbow 3 days before death.

Autopsy Diagnoses: Extensive burns of the left leg and hand; phlegmon of left elbow; and bronchopneumonia of both lower and upper lobes.

Kidneys: Weight 300 gm. each. The gross appearance was characteristic of interstitial nephritis.

Histological Examination: There is severe edema and cellular infiltration, more marked in the cortex than in the medulla, consisting of many polymorphonuclear leukocytes, eosinophils, plasma

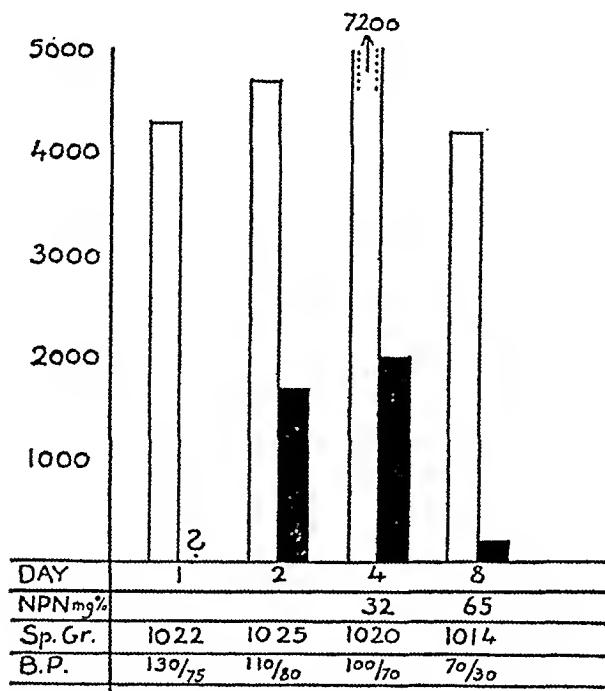


Chart 4. Case 4, A-2073. White column = fluid intake.
Black column = urine output.

cells and monocytes. Tubular dilatation is only moderate in degree. Some albuminous deposit is present but there is no evidence of epithelial degeneration. Glomeruli show questionable intercapillary edema.

Liver: There are focal midzonal necroses of liver cells with an accumulation of polymorphonuclear leukocytes. There is a distinct increase of interstitial infiltration consisting chiefly of polymorphonuclear leukocytes.

Heart: The myocardium shows minute foci of interstitial infiltration consisting of monocytes, polymorphonuclear leukocytes and eosinophils.

Summary: The case is one of severe burns with a high toxic temperature, and in spite of forced intake of fluids (7200 cc. in 1 day), the oliguria increased to almost complete anuria. Although circulatory failure may have contributed to urinary suppression, the specific gravity of the urine fell toward the end and the non-protein nitrogen rose.

The interstitial nephritis, in this case most severe, is of the serous type.

CASE 5. M. R. (A-1989), a negress, 29 years of age, was admitted to the St. Philip Hospital with the chief complaint of severe pain in the right chest. Four days previous to admission she had an attack of pain in the chest, back and neck accompanied by two severe chills with fever, cough and expectoration of blood-tinged sputum. The past history was insignificant.

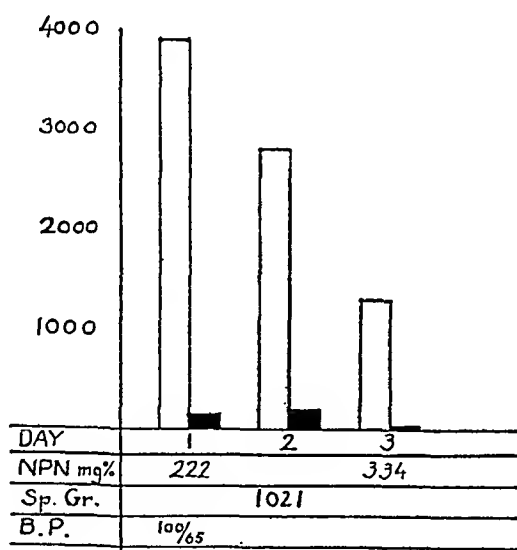


Chart 5. Case 5, A-1989. White column = fluid intake.
Black column = urine output.

Physical Examination: This showed a well developed but poorly nourished female. The symptoms indicated lobar pneumonia involving the right middle and lower lobes.

Course of Illness: The patient became progressively worse and severe jaundice was noted on the 2nd day. Dehydration was apparent and the temperature became subnormal. Death occurred on the 4th day.

Autopsy Diagnoses: Atypical lobar pneumonia, right middle lobe and lower portion of right upper lobe, with abscess formation; hemorrhagic bronchopneumonia, right lower lobe; marked jaundice; and hemorrhagic diathesis.

Kidneys: Weight 240 gm. each. In gross they were characteristic of interstitial nephritis.

Histological Examination: The edema is moderate. There is marked cellular infiltration consisting mainly of large monocytes, lymphocytes, eosinophils and a few polymorphonuclear leukocytes. The infiltration is situated mainly in the cortex. Tubular dilatation is slight and degenerative changes are confined to occasional hyaline droplet degeneration.

Liver: The liver shows slight interstitial infiltration with polymorphonuclear leukocytes.

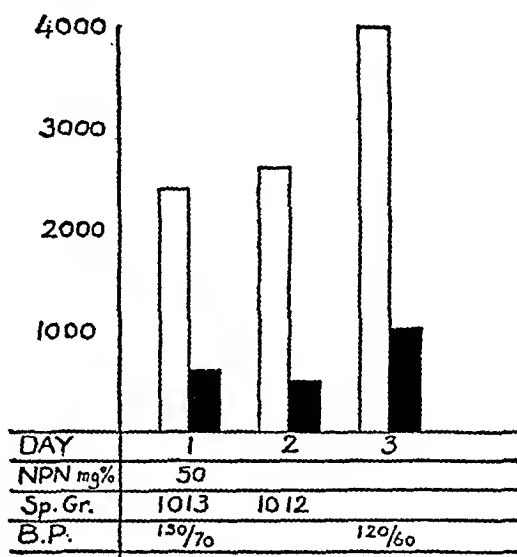


Chart 6. Case 6, A-2013. White column = fluid intake.
Black column = urine output.

Summary: The case is that of an atypical pneumonia with abscess formation and severe jaundice which developed 2 days before death. There was almost complete anuria and a rise in non-protein nitrogen, although circulatory impairment may have contributed to the urinary suppression. It is significant that the urine had a low specific gravity.

The interstitial nephritis is of a more cellular type.

CASE 6. E. W. (A-2013), a negress, 29 years of age, was admitted to the St. Philip Hospital with severe tonsillitis accompanied by general malaise. The onset of illness was 5 days previous. On the night before admission the patient developed pain in the right lower chest and the right upper quadrant of the abdomen. Anorexia was present. Jaundice, associated with itching, developed on the day before admission.

Physical Examination: This showed a well developed but undernourished negress who was markedly jaundiced. The tongue was dry and coated with a raw red border. The tonsils were greatly enlarged and cryptic and the left side of the neck was swollen and tender. The blood pressure was 128/72 mm. Hg. Otherwise the physical examination was negative.

Course of Illness: The patient became progressively worse. The clinical course was characterized by chills, septic temperature and incontinence of urine. Death occurred on the 4th day.

Autopsy Diagnoses: Phlegmon in the soft tissues below the left tonsil and left anterior portion of the neck; thrombophlebitis of jugular vein; multiple pulmonary abscesses; and severe jaundice.

Kidneys: Weight 200 gm. each. In gross they were not remarkable except for a thickened cortex and obscured architecture.

Histological Examination: Marked edema with the infiltrating cells distributed in scattered foci mainly at the medullary cortical junction and consisting of large monocytes, lymphocytes and eosinophils is present. There are a few polymorphonuclear leukocytes. Tubular dilatation is marked, with considerable degeneration of epithelial cells. There are many bile and hemoglobin casts. The glomerular spaces are somewhat dilated.

Summary: The case is that of a septicemia following tonsillitis with thrombophlebitis of the jugular vein and marked jaundice. There was definite oliguria which did not seem to be due to circulatory failure (blood pressure 120/60 mm. Hg.). The specific gravity of the urine was low.

The interstitial nephritis is of the serous type.

Twelve additional cases of interstitial nephritis have also been found during routine autopsies; some of these showed reduced urinary output terminally. The urine, however, was concentrated. In the other cases the clinical observations were not adequate, the patients being moribund when admitted to the hospital. Including all cases, interstitial nephritis was observed under the following conditions:

Five times associated with empyema of the pleural cavity. Isosthenuric oliguria occurred in 1 case in which the non-protein nitrogen, however, did not exceed 50 mg. per cent.

Three times following severe burns (chemical and heat). Isosthenuric oliguria occurred in 2 cases (Cases 2 and 4 reported above).

Three times in the course of septicemia. Isosthenuric oliguria was observed in 1 case (Case 6 reported above).

Twice associated with fever of undetermined origin and jaundice. The cases resembled Weil's disease very closely from both the clinical and the pathological aspects. Spirochetes, however, could not be demonstrated. Oliguria was present in 1 case but was not isosthenuric in character.

Twice in the course of typhoid fever. Oliguria was not observed.

Once following infected abortion and acute appendicitis without septicemia. Hyposthenuric oliguria occurred (Case 1 reported above).

Once following atypical lobar pneumonia. Hyposthenuric oliguria was observed (Case 5 reported above).

Once following nephrectomy of the opposite kidney with oliguria (Case 3 reported above).

In 8 cases the patients were given blood transfusions. Hemoglobin and hematin casts were observed in 5 of these. Two such cases revealed severe histological changes in the kidneys similar to those described in cases of anuria following blood transfusion.

Jaundice was present in 7 cases. The morphological changes in the liver were inconsistent.

MORPHOLOGY

Gross Appearance of Kidney: Interstitial nephritis may be suspected if the kidney is markedly swollen and if the cut surface reveals an obscured architecture of the cortex which is ill defined from the medulla. The surface is mottled and the color varies from brownish red to gray. A grayish streaking of the cortex on the cut surface is often noticeable. The latter is glistening in contrast with the cut surface of the "nephrotic" kidney, which is dull. Grossly interstitial nephritis may closely resemble the picture of early, acute diffuse glomerulonephritis. In fact, it may be indistinguishable from it since punctate hemorrhages on the surface of the kidney and hematuria may occur in pure interstitial nephritis. In many cases, however, no change in the gross appearance indicates its presence.

Microscopic Examination: Several points deserve critical discussion.

(A) *Nature of the Exudate:* This may vary considerably. In

some cases serous effusion into the lymph spaces with scanty, scattered round cells separates the tubules widely. In other instances the cellular exudate predominates. "Serous" interstitial, or "inflammatory edema," as well as the more cellular type appear to be variations of the same condition. Although the serous type is only found in the early acute phase, the interstitial infiltration, even in the beginning, may be predominatingly cellular in character. There is no definite relationship recognizable between the amount of intertubular effusion and the functional disturbances. Although in the majority of cases rapidly terminating in anuric uremia marked swelling and edema of the kidney are present, exceptions occur.

The cellular exudate is composed of polymorphonuclear leukocytes, eosinophils, lymphocytes, monocytes and plasma cells in various proportions. As Lindau²² remarks, these infiltrations sometimes resemble hematopoietic foci containing large cells similar to premature myelogenous cells. I have often found this analogy to be striking. Huebschmann's¹⁷ statement that polymorphonuclear leukocytes predominate in very early phases has not been accepted by other investigators (Koch¹⁹ and Fahr⁸). It is likewise my experience that the nature of the cells present varies with the individual cases independent of the stage of the process. It is a remarkable fact that in the acute phase of inflammation small round cells often by far outnumber the polymorphonuclear leukocytes.

(B) *Distribution of Exudate*: Much emphasis has been placed on the distinction between focal and diffuse interstitial nephritis. The focal distribution has been taken as proof of its bacterial origin. Koch¹⁹ deduces that a toxin would act on the kidney diffusely rather than focally. Fahr⁸ stresses the difference between the two forms because the diffuse exudative type, in contradistinction to the focal, may result in severe functional disturbances and anuria.

In my observation the cellular exudate in both forms is invariably more or less focal in distribution. It is the serous effusion that diffusely penetrates all lymph spaces. The more cellular the type of inflammation, the more "focal" it appears in distribution. The terms focal and diffuse interstitial nephritis in reality indicate the "cellular" and "serous" type of inflammation between which

there is no essential difference. A distinction, therefore, is not justified.

(C) *Differentiation between Hematogenous and Ascending Interstitial Nephritis*: Most of the cellular infiltrations are found at the corticomedullary junction. This is particularly evident in early interstitial nephritis. The cells are crowded in perivascular lymph spaces. As a rule the cortex reveals a much more extensive cellular exudate than the medulla, which may be entirely free from inflammation. In such cases there is no difficulty in distinguishing this type of "hematogenous" interstitial nephritis from the lymphogenic ascending form. The differential diagnosis, however, may occasionally become exceedingly difficult if the process is diffusely distributed throughout the cortex and medulla and involves the peripelvic tissue. Pyelonephritis does not always ascend within the tubules; it may spread within the lymph channels (see Putschar²⁷ for literature). Cases have been observed in which severe interstitial nephritis with anuria developed some time after the other kidney had been removed because of calculi and ascending pyelonephritis (Fahr,⁸ and Case 3 reported above). In order to establish the hematogenous origin in such instances ureteritis and pyelitis must be ruled out by special examination.

(D) *Involvement of Glomeruli*: Interstitial nephritis may occur in association with diffuse glomerulonephritis. With this exception the glomeruli appear normal, although they may be engorged. The glomerular space is often distended with fluid containing some coagulated plasma and occasionally minute hemorrhages are observed. It must be emphasized, however, that this distention does not involve all the glomeruli and it may be entirely absent in complete anuria where a great number of casts are found in the tubules. Garloch and Klein¹⁰ have described a serous intercapillary glomerulitis in 1 case of interstitial nephritis. In my experience slight swelling and edema of intercapillary connective tissue framework of the glomeruli may be observed occasionally. The lesions in these cases, however, were not noteworthy, with the exception of 1 case in which the intercapillary edema was even accompanied by an accumulation of polymorphonuclear leukocytes between the capillary loops. The intertubular capillaries are invariably markedly engorged and minute hemorrhages are frequently encountered.

(E) *Involvement of Tubular Apparatus:* Tubular changes occur almost invariably. In most instances there is a diffuse though moderate degree of dilatation of the lumens. This is particularly marked in cases of the acute "serous" type of inflammation. The tubules contain coagulated, slightly pinkish staining material. In the more cellular and less edematous cases dilatation may be absent. Degeneration of epithelial cells, as a rule, is slight and appears to be within the range of reversible vacuolic, albuminous or hyaline droplet degeneration of the cytoplasm. Under certain conditions, however, the regressive metamorphosis may be so severe as to resemble the tubular necrosis seen in mercury poisoning. Such changes are obviously irreversible, as evidenced by calcification and epithelial regeneration. Cases of this type have been described by Kuczynski²⁰ and recently by Goldring and Graef.¹¹ Their descriptions indicate the presence of interstitial nephritis in addition to the tubular degeneration.

As a rule, the renal tubules remain intact. Occasionally, however, the interstitial infiltration may break through the basement membrane, destroying the epithelial cells and entering the lumen. Polymorphonuclear leukocyte casts are therefore not infrequently encountered.

Other accessory findings, such as bile pigment in epithelial cells, bile casts, hemoglobin and hematin casts, depend on the concomitant or precursory condition causing interstitial nephritis. A parallelism between tubular dilatation, epithelial degeneration and functional disturbance cannot be established.

CONDITIONS CAUSING INTERSTITIAL NEPHRITIS

Infectious Diseases and Septicemia: Interstitial nephritis is known to accompany almost every infectious or septic condition. So frequently is it observed in scarlet fever and Weil's disease that it is regarded as a characteristic complication. Measles, diphtheria, typhoid fever, variola and pneumonic infections are listed among the precursory conditions. It is found also occasionally in tuberculosis, and Fahr⁸ assumes that in such instances superimposed streptococcic infections should be taken into account.

The question has often been discussed as to whether the bacteria themselves or their toxins should be held responsible for

these renal changes. It is true that microorganisms sometimes can be found in the kidney, but their demonstration is of little significance. They may circulate throughout the body in instances of septicemia or bacteriemia without causing local reaction. Moreover, a search for bacteria in the kidney in cases of interstitial nephritis has often been in vain (Munk,²⁴ Kuczynski,²⁰ and others).

Whether bacteria or their toxins are looked upon as injuring agents, the allergic reaction to various antigens is accepted as the most important factor. Interstitial nephritis is regarded as the reaction of the hypersensitive kidney to either bacteria (Koch¹⁹) or their toxins (Fahr,⁸ and Huebschmann¹⁷). It should indeed be emphasized that it is the tissue reaction that determines this renal lesion rather than any one specific agent. Interstitial nephritis may even occur without bacterial infection.

Conditions Associated with Hemolysis: Various conditions associated with hemolysis may result in interstitial nephritis. This has frequently been described in anuric uremia following blood transfusion with "compatible" blood. Although most authors have paid much more attention to the formation of hematin casts and tubular degenerative changes, interstitial edema and inflammatory infiltration have frequently been emphasized. The serous type of interstitial nephritis is described in Witts's³² report of such a case. Lindau²² stresses more the cellular infiltration. Bordley,⁴ reviewing the literature on this subject, finds interstitial cellular infiltration mentioned in 6 out of 9 autopsied cases. Goldring and Graef¹¹ gave convincing evidence of edematous interstitial nephritis in their cases of death following blood transfusion. Baker³ lists a varying amount of edema and possibly "chronic inflammatory infiltration" of interstitial tissue among the findings in the kidney in urinary suppression following blood transfusion. He considers these changes to be a late result because they were absent in 1 of his cases which came to autopsy 4 days after transfusion. From a review of the literature, however, it remains uncertain whether or not interstitial nephritis accompanies all instances of anuria after blood transfusion.

One would expect to find similar conditions in black water fever. Autopsy reports, however, do not list interstitial nephritis among the morphological findings. The one histological section of such a

case I had an opportunity to study revealed considerable interstitial infiltration with round cells and some edema.* This lesion may have been neglected in view of the more impressive changes in connection with the hemoglobinuria.

Hepatorenal Syndrome: Interstitial nephritis may, furthermore, occur in what is called the hepatorenal syndrome. It is not my intention to analyze this still somewhat cloudy clinical concept, which comprises a variety of essentially different conditions. I refer at present merely to cases in which impairment of renal function, or better oliguria, anuria and rise of non-protein nitrogen in the plasma follow an acute liver injury. For our purpose it is necessary to distinguish between two types of cases — those in which the preceding liver lesion is of an infectious nature, and those in which the liver injury is non-infectious.

If interstitial nephritis is found to be associated with cholangitis and cholangiolitis, or an interstitial hepatitis, bacteria or their toxins may ultimately be held responsible for the renal lesions as in any other infectious disease.

However, cases are recorded in which "renal involvement" resulted from non-infected liver injuries. Helwig and Orr¹³ and Helwig and Schutz¹⁴ reported cases of traumatic liver necrosis in which death occurred in anuric uremia. As a "striking feature" they describe medullary foci of interstitial infiltration with mononuclear cells, eosinophils and plasma cells. Lichtman and Sohval²¹ noted acute inflammation of the stroma in addition to tubular degeneration in a case of anuric uremia following subacute yellow atrophy of the liver. The authors interpreted the interstitial infiltration as a reaction to tubular degeneration. This, however, is not tenable in view of the fact that the much more severe tubular degeneration in mercury poisoning is not associated with interstitial infiltration except where there is calcification of epithelial debris, in which case occasional polymorphonuclear cells are found in their immediate vicinity (Fahr⁸). Furthermore, it should be considered that a medullary interstitial infiltration is not likely to result from tubular degeneration in the cortex especially since this region is free from local inflammatory reaction. Thus, irrespective of the interpretation of its pathogenesis we may state that inter-

* I am indebted to Captain DeCoursey, Army Medical Museum, for kindly supplying me with this section.

stitial nephritis is found in hepatorenal syndrome uncomplicated by bacterial infection.

Finally, we have found serous interstitial nephritis mentioned in cases of merely toxic, non-bacterial food poisoning. Nonnenbruch²⁵ describes such an observation and refers to a similar report by Eppinger.

CORRELATION OF FUNCTIONAL DISTURBANCES WITH INTERSTITIAL NEPHRITIS

Interstitial nephritis may be associated with oliguria, anuria, rise in non-protein nitrogen, and lack of renal concentration power. On the face of it, the urinary suppression appears to be renal in origin. This question is still under discussion and various theories have been advanced to explain the anuria.

Renal Edema and Anuria: Edema and increased intracapsular pressure, in particular on the tubular apparatus, have been held responsible for urinary suppression. This explanation deserves serious consideration in view of the fact that urinary secretion may be restored immediately after decapsulation. However, Koch, who examined a biopsy of a kidney, and also autopsy material from a case of postscarlatinal interstitial nephritis associated with anuria, came to the conclusion that the edema does not cause the anuria. He found that the tubules are of normal caliber and that the glomerular spaces are not dilated. It is generally true that the distention of glomerular spaces and tubules is a very variable finding. It should be consistent and marked, if edema causes the anuria, by pressure on the tubular apparatus. Equally important is the fact that the gross swelling of the kidney may be negligible or absent in interstitial nephritis with anuria. The temporary restoration of diuresis after decapsulation therefore requires another explanation.

Hemoglobin Casts and Anuria: In interstitial nephritis associated with hemoglobinuria, as in black water fever or after blood transfusion, the anuria has been explained mechanically. This theory, advanced by Baker and Dodds,² assumes that hemoglobin is precipitated in the tubules by acid urine which contains a sodium chloride concentration exceeding 1 per cent. The hemoglobin is converted into hematin and is supposed to block the urinary outflow. The authors have shown experimentally that this

precipitation and the anuria can be prevented if the urine is kept alkaline. It is, however, questionable whether their experiments on rats, which were later confirmed on dogs, can be applied to human beings. Furthermore, the tubular dilatation is variable in degree and distention of Bowman's capsule may even be absent. In their experiments with repeated hemoglobin transfusions and acid diet in dogs DeGowin, *et al.*,⁷ succeeded in producing hematin casts in the collecting tubules "so arranged as to block the tubules completely." The majority of their dogs, however, excreted a volume of urine which "continued to be fairly high until death occurred." Hence, the presence of hemoglobin casts does not prove that they actually cause urinary suppression by mechanical blockage. In this connection it is of interest to notice that Case 2 (reported above) showed seemingly complete blockage of collecting tubules with hemoglobin casts, and yet the urinary volume was restored to normal.

Although the possibility of mechanical blockage of descending tubules by hematin casts cannot be denied, it appears at least doubtful whether such a process can be extensive enough to cause anuria.

Circulatory Disturbances and Anuria: Disturbance of circulation has been cited as the cause of anuria. We have to distinguish between local (renal) and general impairment of circulation. Each may play an important part. Many patients with acute anuria following blood transfusion, burns, hepatic injuries, and so on, die under conditions resembling shock. Falling blood pressure, indicating the collapse of peripheral circulation, furnishes reasonable explanation for the reduced urinary output. Provided the kidney function is normal, one should under such circumstances expect a highly concentrated urine. It has, however, frequently been pointed out that isosthenuria or hyposthenuria is characteristic of this type of case and it has, therefore, been concluded that the impairment of renal function is the ultimate cause of oliguria. This assumption, in my opinion, is only partially correct. The mechanism may be interpreted as follows: diminished peripheral circulation results in reduced capillary pressure and reduced glomerular filtration. A diminished oxygen supply to the epithelial apparatus, on the other hand, causes reduction of their function. Hence, less water is reabsorbed. Thus, oliguria and decreased

concentration power from this point of view are to be looked upon as parallel phenomena, both resulting from insufficient peripheral circulation.

This explanation, however, does not apply to those cases in which there is no impairment of circulation. These cases (for instance Cases 1, 3 and 6, reported above) show normal or increased blood pressure during the period of marked oliguria with low specific gravity of the urine. One may think of locally (renal) impaired circulation, which may be brought about by increased intrarenal pressure, to be due to edema. One may also consider the possibility of vascular spasm. Hesse and Filatov¹⁵ have given experimental evidence of arterial spasm in the kidney in hemolytic shock. The same theory is advanced to explain the anuria following liver injury (Furtwaengler,⁹ and Pytel²⁸). A satisfactory explanation has not as yet been given.

Renal Changes and Anuria: It is noteworthy that there is no consistence in regard to the evidence on which the various theories of the renal origin of anuria are based. Anuria of the isosthenuric or hyposthenuric type with a rise in non-protein nitrogen may be associated with various degrees and types of renal changes. Cellular infiltration, edema, hematin casts, tubular dilatation and epithelial degeneration may be present simultaneously, but any one of these processes may be absent. Thus, it seems more likely that these morphological changes are more of an accessory nature and that they are to be looked upon as a reflection of extrarenal disturbance not causing the anuria but accompanying it.

In fact, morphological changes in the kidney may be entirely absent in anuric uremia. It is not our purpose to discuss the various conditions in which "extrarenal uremia" with anuria occurs. If we intend, however, to evaluate the significance of structural changes in the kidney in regard to their function we must bear in mind that no parallelism can be established. Neither one of the above mentioned changes in the kidney fully explains the renal origin of anuria.

There is even uncertainty as to whether any of the functional disturbances necessarily indicates renal damage. The oliguria, if not caused by circulatory failure, may be due to what Lichtman and Sohval²¹ called prerenal deviation of water. The hyposthenuria may be both renal and extrarenal in origin, as Nonnen-

bruch²⁵ and Weiser³⁰ pointed out. Function tests may reveal only slight impairment of function (Cooper⁶).

Hence, we conclude that sufficient evidence has not been presented to prove the renal origin of anuria and uremia in acute interstitial nephritis and associated conditions on the basis of morphological changes. Pure functional disturbances of circulation are more likely to be its cause.

CONCEPT OF INTERSTITIAL NEPHRITIS

It is reasonable to conclude that acute interstitial nephritis represents an accessory local manifestation of a general reaction of the body to a toxic protein split product. More specifically, I regard it as a hyperergic reaction. The toxic substances, which I assume to be protein split products, may result in various injuries to parenchymatous organs. It may cause a shock-like condition and produce anuric uremia. If the kidney tissue is hypersensitive to this protein split product a non-specific hyperergic reaction takes place, the manifestation of which is the interstitial nephritis.

I have pointed out above that most authors discussing interstitial nephritis in infectious conditions interpret it as a hyperergic reaction to bacteria and their toxins. Although bacteria seem to play a most important rôle in the production of interstitial nephritis, in many cases they are not essential.

There seems to be sufficient evidence that acute interstitial nephritis can be produced experimentally by repeated injections of any non-bacterial antigen such as serum or egg white. Longcope,²³ who attempted to produce a prolonged anaphylactic shock in animals, observed marked diffuse or focal interstitial nephritis. Ahlström,¹ working with bacterial toxins and serum, specifically designates the interstitial nephritis as a non-specific allergic reaction of the kidney. The complete literature on this subject can be found in Horn's paper¹⁶ on the experimental nephropathies.

Interstitial nephritis, on the other hand, is found to be accompanied by conditions we do not ordinarily associate with allergy, such as acute severe hemolysis, concomitant or antecedent liver damages, burns, and so on. It is not invariably present in such cases and if so varies considerably in degree. If it occurs, it indicates, in my opinion, that an allergic factor is involved. This

concept is in keeping with the interpretations given to any one of the various conditions in question. In fact, it is strongly supported by a number of theories and facts, some of which will be pointed out.

So-called hemolytic shock following blood transfusion with "compatible" blood may have characteristics resembling anaphylactic reaction (Bordley,⁴ and Witts³²). Urticarial rash, for instance, may appear immediately after transfusion. The hemolysis, though usually present, may be absent in fatal cases (Hancock¹²). That hemolysis is not an indispensable factor has been shown by Petroff and Bogomolova,²⁶ who experimentally reproduced an identical condition with plasma alone. The urinary suppression, in particular, does not parallel the hemolysis (Iljin¹⁸), but rather depends on protein split products liberated from hemolyzed red blood cells. It is the protein and its split products which may, though not in all cases, result in an allergic hyperergic reaction. The same is true of various conditions associated with severe hemolysis, such as black water fever which has frequently been interpreted as an allergic phenomenon.

Anuria in so-called hepatorenal syndrome is likewise believed to be the effect of foreign proteins or their split products which are either liberated from destroyed liver tissue or passing undetoxified from the intestine through the functionally impaired liver into the circulation (Boyce and McFetridge,⁵ Helwig and Schutz,¹⁴ Pytel,²⁸ Koch,¹⁹ Rosenbaum,²⁹ and Wilensky and Colp³¹).

Thus, the common denominator for all conditions in which we encounter interstitial nephritis is the foreign protein or protein split product derived from bacteria, their toxins, destruction of cells, destruction of erythrocytes, or possibly inadequately assimilated protein absorbed through the intestine.

Its effect depends on its nature and the state of reaction of the body cells. In the first place, it may result in an acute or prolonged shock-like condition. Various purely degenerative lesions may be encountered in the kidney and the liver. Attention has largely been focused on regressive changes in the tubular epithelial cells of the kidney in severe hemoglobinuria. They are described as necrotizing nephroses, whereby excretion or reabsorption of hemoglobin may be a contributing factor to the degeneration

of epithelial cells. In fatal cases of so-called hepatorenal syndrome they are believed to be the result of the specific action of hepatogenic toxic substances. Focal necrosis in the liver and tubular degeneration in the kidney, however, are such common findings under such a multitude of circumstances that they hardly bear specific significance.

In the second place, foreign proteins and protein split products may act as an antigen. Permanent or periodic discharge will in time result in an allergic reaction, which may set forth inflammatory changes in various tissues (hyperergic reaction). The interstitial nephritis is the most prominent manifestation; interstitial hepatitis, myocarditis, pancreatitis and adrenalitis are less frequently encountered. Thus, I would not expect interstitial nephritis to appear at too early a date, an assumption that well coincides with actual observation. Kuczynski²⁰ reports an interesting case of scarlet fever in which death occurred as early as 2 days after onset of the rash. Although the kidneys were markedly edematous and revealed hyperemia of the cortex, and cellular interstitial infiltration was absent, the tubular epithelial cells showed degeneration.

In the cases of hepatorenal syndrome purely degenerative changes are noted if death occurs in 24 to 48 hours. Interstitial nephritis is observed in those instances in which 5 to 7 days elapse after the liver injury (operation) before the patient dies. The same apparently is true in fatalities following blood transfusions, and prompted Baker's³ statement that edema and interstitial infiltration should be looked upon as a "late result."

It becomes clear from this point of view that oliguria or anuria and interstitial nephritis are coincident rather than causatively connected, both resulting from foreign protein or protein split products. Although they must not necessarily be present simultaneously, we are not surprised to find them frequently associated. Such coincidences, however, do not justify the separation of interstitial nephritis with hyposthenuric oliguria as a disease entity.

NOTE: I wish to acknowledge my indebtedness to my medical and surgical colleagues who have so kindly allowed me the free use of their clinical records.

SUMMARY AND CONCLUSIONS

1. Six cases of acute hematogenous interstitial nephritis are presented, all of which show more or less marked isosthenuric oliguria or anuria and a rise of non-protein nitrogen in the blood. Twelve other cases of interstitial nephritis, which were found incidentally at autopsy, are included.

2. The morphological characteristics of this lesion are described. It is emphasized that even in the early stage the interstitial exudate contains plasma cells, lymphocytes, eosinophils and monocytes, in addition to polymorphonuclear leukocytes. A distinction between focal and diffuse interstitial nephritis should not be made.

3. The conditions causing interstitial nephritis are listed. In addition to the infectious diseases and septicemia, it is found to follow conditions associated with hemolysis, in particular blood transfusion with incompatible blood. It is also found in the hepatorenal syndrome with infectious and non-infectious liver injuries.

4. The correlation of functional disturbances with interstitial nephritis is discussed. It is concluded that the anuria does not result from renal edema by pressure on the tubular apparatus, neither is it caused by blockage of tubules if associated with hemoglobinuria. General or local renal circulatory disturbances are held to be the most likely cause of oliguria and lack of concentration power.

5. Hematogenous interstitial nephritis is regarded as an allergic hyperergic reaction to foreign proteins or protein split products coincidental rather than causatively connected with hyposthenuric oliguria and anuric uremia.

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THE PHARYNGEAL PITUITARY GLAND *

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Accessory or aberrant nodules of the endocrine tissues are not an infrequent finding. Notable examples are the lateral aberrant thyroid, the lingual thyroid, and adrenal cortical tissue beneath the capsule of the kidney. In most instances the small masses are found along the pathway of developmental migration of the main organ.

The anterior lobe of the pituitary gland is derived from an epithelial evagination of the roof of the posterior nasopharynx, known as Rathke's pouch. This mass of epithelial cells loses its attachment to the pharynx, migrates through the tissue which later becomes the body of the sphenoid bone, and comes to rest in the anlage of the sella turcica. Small masses of pituitary tissue may occasionally be found in the body of the sphenoid bone but according to the investigations of Haberfeld¹ and Christeller² there always remains a small piece of typical or atypical pituitary tissue in the pharyngeal mucosa that has been designated as the pharyngeal pituitary gland.

METHODS

Material from 54 autopsies at the New York Hospital during the period from July 1936 to May 1937 was used. In order to avoid autolytic changes only those cases in which the autopsy was performed a few hours after death were chosen for study. Otherwise there was no selection of the specimens.

The base of the skull was exposed by removal of the calvarium and brain. A coronal cut just anterior to the optic groove was made with a chisel through the body of the sphenoid bone and the bony nasal septum into the posterior nasopharynx. From each lateral extremity of this incision sawcuts through the thickness of the base of the skull were extended posteriorly and brought to taper in the occipital bone at a point near the foramen magnum.

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After fixation in 6 per cent formaldehyde the pituitary gland was dissected free, weighed and measured, and then cut into four horizontal blocks. One paraffin section from each block was stained with Mallory's aniline blue collagen stain. The mucosa and periosteum covering the vomer were stripped from the bone as far as the junction of the vomer and the sphenoid. The firm fibrous tissue and vascular channels that enter the periosteum at the vomerosphenoidal articulation were cut across and the firmly adherent periosteum was dissected from the remainder of the nasopharyngeal surface of the vomer. A sagittal block from the midline, centered on the vomerosphenoidal articulation, approximately 3 mm. in lateral dimension and 15 to 25 mm. in anteroposterior dimension, was prepared and embedded in paraffin. Alternate successive groups of 5 and 10 sections each were saved and discarded respectively until the entire block was cut. The first section of each group of 5 was stained with hematoxylin and eosin. The additional sections were used for staining with Mallory's aniline blue collagen stain, Krause's differential stain, and van Gieson's method. A total of over 8800 sections was mounted.

The greatest anteroposterior and cephalocaudal dimensions were determined with an ocular micrometer. The greatest lateral dimension was computed from the number of sections in which the pharyngeal pituitary appeared.

In this paper the term pituitary gland will be used in the ordinary sense to designate the structure contained in the sella turcica, and the term pharyngeal pituitary gland to designate the pituitary-like structure located in the mucosa of the nasopharynx.

FREQUENCY, LOCATION, SHAPE AND SIZE

The pharyngeal pituitary gland was found in 51 of the 54 cases studied. It was most constantly located in the midline deep in the mucosa or in the periosteum beneath or near the vomerosphenoidal articulation (Fig. 1). It occurred most frequently as a single, well circumscribed and encapsulated structure. In a few instances irregular cords or islands of cells extended into the surrounding tissue. The general shape was that of a flattened prolate spheroid.

Haberfeld¹ studied the pharyngeal pituitary in 51 cases and concluded that most of the growth of the gland occurred in the fetus and during the first few months of life, and that there was little or

no growth thereafter. A study of the size of the pharyngeal pituitary gland in the present series confirmed this conclusion (Table I). An analysis of variance of the age of the individuals (decades) and the size (sum of the three dimensions) of the pharyngeal

TABLE I

Sum of the Three Dimensions (Millimeters) of the Pharyngeal Pituitaries of Individuals in Cases Studied in Relation to Age

Less than 1 mo.	1 mo.-1 yr.	1-10 yrs.	11-20 yrs.	21-30 yrs.	31-40 yrs.	41-50 yrs.	51-60 yrs.	Over 61 yrs.
0.53	3.01	1.38	1.97	1.31	1.05	1.17	0.67	1.84
0.74	5.36	2.51	2.14	1.91	3.69	2.41	2.41	2.64
1.23	6.93	3.87	4.35	2.06	4.19	3.66	2.73	4.73
1.87			4.71	3.81	5.67	3.82	3.15	
1.98			5.48	6.52	6.25	3.89	3.95	
3.14			8.12		6.55	4.75	4.78	
					7.12	5.33	4.90	
							5.32	
							5.43	
							5.46	
							6.44	

pituitary showed that there was as much variation of size within the decades as between the decades (Table II). The lack of progressive growth of the pharyngeal pituitary is in contrast with the increase in size of the pituitary gland which is rapid in the first

TABLE II

Analysis of Variance of Sum of Three Dimensions of Pharyngeal Pituitaries Studied and Age by Decades

Source of Variation	Degrees freedom	Total squares	Mean square
Total	50	187.14	3.74
Between	8	35.96	4.49
Within	42	151.18	3.60

F = 1.25 — not significant.

decade and progresses gradually through puberty into adult life.

The largest pharyngeal pituitary gland observed in this series occurred in a girl 15 years of age and measured 6.62 mm. in length, 1.15 mm. in width, and 0.35 mm. in depth. The smallest gland was in a newborn infant and measured 0.22 mm. in length, 0.21 mm. in width, and 0.10 mm. in depth.

HISTOLOGICAL FEATURES

The pharyngeal pituitary glands were composed essentially of undifferentiated epithelial cells and differentiated cells similar to those in the anterior lobe of the pituitary gland.

Undifferentiated epithelium was present in 32 of the 51 cases. It was usually of the transitional type but in 1 instance there were definite intercellular bridges. Keratinization and keratin granules were not observed. The undifferentiated cells were arranged in small nests with an indefinite basal layer. The relative amount of undifferentiated epithelium in each case in relation to age is shown in Table III.

TABLE III

Relative Amounts of Undifferentiated Epithelium in Pharyngeal Pituitaries with Ages in Years of Individuals

None		One plus	Two plus	Three plus	Four plus
1	12	38	14	15	1
1	22	52	17	37	1
1	22	55	20	45	16
1	30	57	32	46	24
1	30	69	47	55	34
1	40		50	58	35
1	40		53	59	41
1	51		59	80	49
4	54		59		55
6			72		

Statistical evaluation of these figures by the method of analysis of variance showed that there was greater variation within the groups than between the groups. The insignificant correlation between the amount of epithelium and the age of the patient indicates that there is no progressive differentiation of the cells as is found in other functional organs.

In 3 cases there was cyst formation within the transitional epithelium and slight infiltration with polymorphonuclear leukocytes. In 10 cases there were glandular acini lined by columnar or cuboidal cells. The lumens contained an acidophilic homogeneous substance. In 7 of the 10 cases the acini were associated with nests of transitional epithelium.

The differentiated tissue in the pharyngeal pituitary had the same histological appearance as that in the pituitary gland (Fig. 2),

but there were conspicuous quantitative differences. An approximation of the relative numbers of acidophilic and basophilic cells in the pharyngeal pituitary is shown in Table IV.

With the exception of the acidophilic cells in 6 cases there was a conspicuous deficiency of basophilic and acidophilic cells. In 17 and 18 cases the acidophilic and basophilic cells respectively were entirely absent, and in 13 both were absent. In 7 of the 13 instances in which chromophilic cells were absent, chromophobic cells were also absent. Thus, in 25 per cent of cases there were no chromophilic cells and in 35 per cent either the acidophilic or basophilic cells were absent. Even when chromophilic cells were present they were few in number and constituted less than 1 per cent

TABLE IV
Relative Numbers of Chromophilic Cells in Pharyngeal Pituitaries

	Acidophilic cells	Basophilic cells
None	17	18
One plus	11	24
Two plus	15	8
Three plus	2	1
Four plus	6	0
Total positive	34	33
Total	51	51

of all cells. The relative deficiency of chromophilic cells and the small size of the entire gland render it unlikely that the pharyngeal pituitary contributes any significant part to pituitary function.

Colloid was frequently associated with the chromophilic cells and was present as a conspicuous element in 11 cases. This colloid was for the most part fuchsinophilic in the classification of Kraus³ and probably represented degenerating cells. In 3 of the 11 cases there was also conspicuous colloid in the pituitary gland but the condition in the two locations was not closely correlated.

The basophilic cells were vacuolated in 2 cases and the eosinophilic cells in 1 case. Vacuolization of the basophils is usually accepted as an index of function and upon this basis secretory activity must be accepted. The analogous cells in the pituitary were in all 3 instances vacuolated.

The interstitial tissue and vascular supply of the pharyngeal pituitary were essentially the same as in the pituitary. There were

delicate strands of connective tissue and numerous thin walled capillaries. Haberfeld¹ reported a progressive increase in the stroma with advancing age, but this was not apparent in the present series, nor was it noted in the cases studied by Christeller.²

In 1 case the pharyngeal pituitary was associated with a small lymph node. In most cases there were numerous myelinated nerves and large vascular sinusoids in the surrounding tissue.

In 3 cases, individuals 1, 14 and 24 years old respectively, the pharyngeal pituitary gland was located immediately beneath the epithelial cells of the pharyngeal mucosa, and in an additional 2 cases, a stillborn infant and a 69 year old male, there was actual union of the hypophyseal and mucosal cells (Fig. 3).

SPECIAL CASES

Of particular interest were a number of cases in which the pituitary gland showed pathological changes: (1) a female, 34 years old, who died of cerebral hemorrhage 16 hours postpartum at term; (2) a boy, 16 years old, with an adamantinoma of the sellar region and apparently complete compression atrophy of the pituitary; (3) a girl, 12 years old, with Addison's disease and absence of basophilic cells in the anterior lobe of the pituitary; and (4) a male, 32 years old, with widespread metastases from a teratoma of the testis.

The cases of pregnancy and teratoma of the testis may be considered together. In a physiopathological analysis of diseases of the pituitary Erdheim⁴ concluded that all conditions associated with urinary excretion of prolan result in the same alterations in the pituitary gland, namely the appearance of the so-called "pregnancy cell." Pregnancy cells⁵ are about the same general size or slightly smaller than acidophilic cells. They tend to a columnar shape and palisade arrangement, are vacuolated at the ends, have a stringy reticulated cytoplasm and contain a few acidophilic granules. In the pharyngeal pituitary from the postpartum female transitional epithelium (Fig. 4) was the only cellular type present. It should be pointed out that the epithelial cells in this case are larger and the cytoplasm is less chromatic than in any other case. In the patient with a teratoma of the testis there were abundant acidophilic cells in the pharyngeal pituitary. The acidophilic cells were moderately well granulated and could not be identified

as "pregnancy cells." This patient was known to be excreting large quantities of prolactin and the failure of either hypertrophy or the appearance of pregnancy cells in the pharyngeal pituitary is strong evidence of lack of response to an adequate stimulus. The significance of the abundant acidophilic cells associated with teratoma of the testis is difficult to evaluate because in 5 other cases the acidophilic cells were conspicuous and not related to an endocrine disturbance. Christeller² reported a case of Froehlich's syndrome associated with destruction of the pituitary and abundant eosinophilic cells in the pharyngeal pituitary. In the 5 pharyngeal pituitaries from pregnant females studied by Haberfeld¹ there was no distinctive feature.

In the patient with an adamantinoma of the hypophyseal stalk and apparent destruction of the entire pituitary gland the pharyngeal pituitary was again composed of undifferentiated epithelium and no deductions on possible compensatory hypertrophy are possible. Female distribution of hair, general adiposity and hypoplasia of the genitalia in this 17 year old boy support the diagnosis of disturbed pituitary function. Christeller² reported 2 cases of neoplastic compression of the pituitary in which the pharyngeal pituitary was composed entirely of undifferentiated epithelium.

Kraus⁶ first noted that in Addison's disease there is a conspicuous decrease or absence of basophilic cells in the anterior lobe of the pituitary. In the 1 case of Addison's disease in this series basophilic cells were entirely absent in the pituitary but in the pharyngeal pituitary there were a very few definite basophilic cells with vacuoles. The presence of these basophilic cells is further evidence that cells in the pharyngeal pituitary of similar morphological appearance to those in the pituitary do not react to the same stimuli.

DISCUSSION

A critical evaluation of the morphological findings in terms of physiological function is difficult because of the limited number of observations of the pharyngeal pituitary in examples of known disturbances of the pituitary gland. The lack of significant change in the pharyngeal pituitary in 5 pregnant females reported by Haberfeld¹ and the one in this series indicates that the cells do not respond to the altered hormonal conditions of pregnancy. The

failure of compensatory hypertrophy in the 2 cases of neoplastic compression of the pituitary observed by Christeller² and the 1 similar case in this series is further evidence of an inability to respond to an adequate stimulus. Finally, there is no evidence of progressive growth and differentiation that is observed in all other endocrine glands.

The case reported by Christeller² and 1 in the present series in which there are conspicuous eosinophilic cells in the pharyngeal pituitary associated with Froehlich's syndrome and teratoma of the testis, respectively, indicate possible physiological activity. There is also the minor histological evidence of function as shown by occasional vacuolization of the chromophilic cells.

SUMMARY AND CONCLUSIONS

A small mass of typical or atypical pituitary tissue was found in the pharyngeal mucosa in 51 of 54 unselected cases examined. The differentiated cells in the pharyngeal pituitary gland are histologically identical with those in the anterior lobe of the pituitary gland but there are relatively few chromophilic cells.

Under normal conditions of growth and activity it is unlikely that the pharyngeal pituitary gland contributes any significant physiological function, but in some cases of altered structure or activity of the pituitary gland it cannot be denied that the pharyngeal pituitary gland may undergo structural alterations and serve as an endocrine organ.

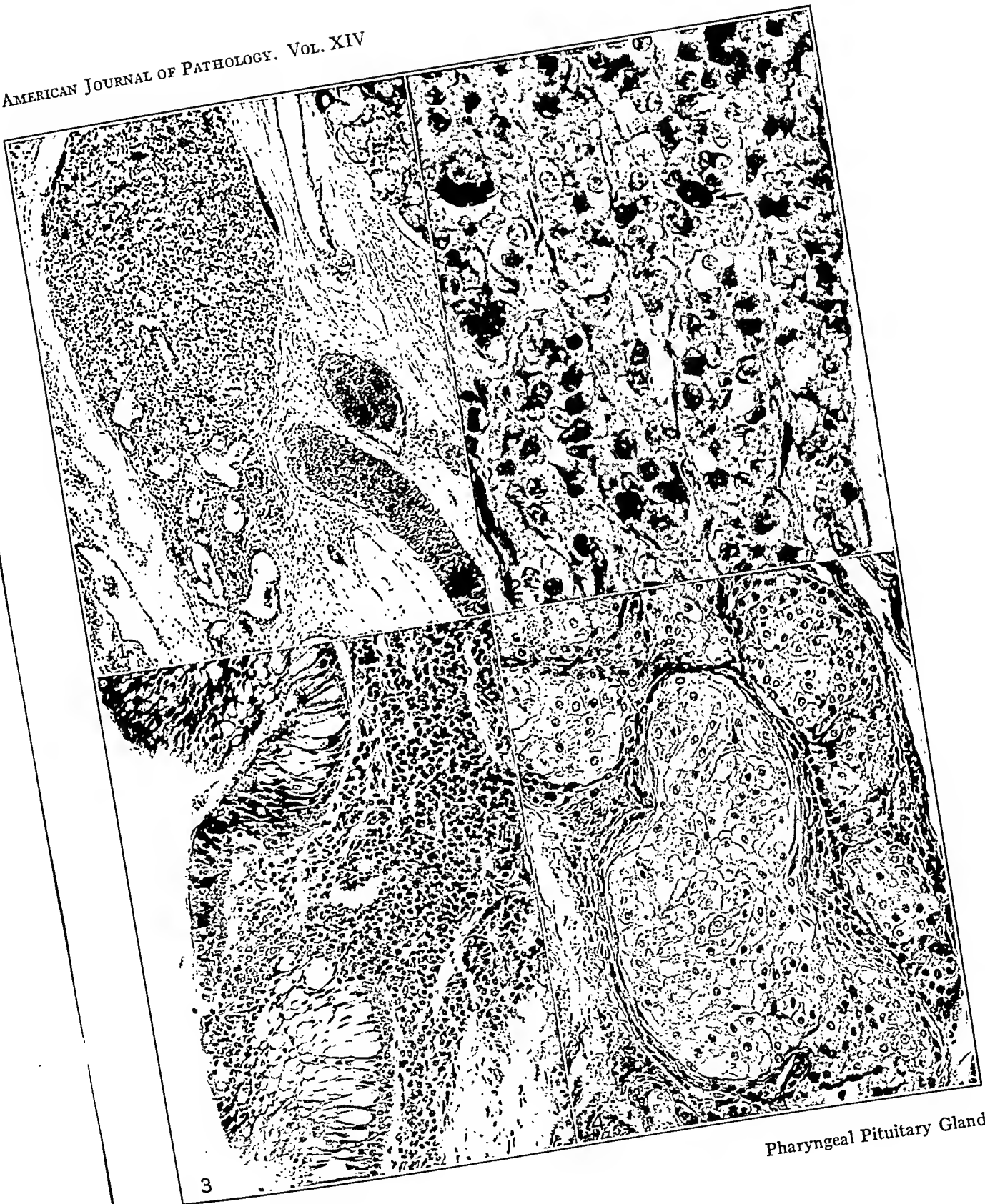
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DESCRIPTION OF PLATE

PLATE 145

- FIG. 1. A pharyngeal pituitary gland composed of differentiated cells and a few glandular acini. Note the mucosal glands of the pharynx at one side and the large vascular spaces in the surrounding fibrous tissue.
- FIG. 2. A high power microphotograph to show the appearance of the differentiated cells of the pharyngeal pituitary. The arrow points to an acidophilic cell.
- FIG. 3. A microphotograph to show union of the pharyngeal pituitary with the epithelial cells of the mucosa of the pharynx.
- FIG. 4. Swollen transitional epithelium in the pharyngeal pituitary associated with pregnancy.



3

Melchionna and Moore

Pharyngeal Pituitary Gland



DIFFERENCES BETWEEN CASTRATION CELLS AND THYROID-ECTOMY CELLS OF THE PITUITARY OF THE RAT IN RESPONSE TO THE ADMINISTRATION OF ESTRONE AND THYROID EXTRACT *

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In previous experiments I have made attempts to identify the histological types of cells in the pituitary which produce the various pituitary hormones. Histological differences have been described between the cells in the rat's pituitary which react to thyroidectomy and the cells which react to castration^{1,2}; differences have been described in the manner in which the adrenals react to these two operations^{3,4}; variations in the weight of the pituitary after these operations have been recorded²; and I have suggested that thyroidectomy cells and castration cells probably produce different hormones,⁵ and that the adrenocorticotrophic hormone is unrelated either to thyroidectomy cells or to castration cells.⁴ These structural and functional differences have led me to regard these cells as having more differences than similarities. In fact, thyroidectomy cells in histological appearance in some ways seem more like chromophobes than basophils, in so far as the cytoplasm stains with difficulty and contains only fine granules.²

On the other hand, some investigators have regarded castration cells and thyroidectomy cells as identical. Severinghaus, Smelser and Clark⁶ described the histological changes in 9 thyroidectomized rats as "typical castration cells" and said that the "basophiles of the thyroidectomized rats are similar to those of castrate and thyroid-treated rats." The fact that the testes do not atrophy in thyroidectomized rats³ indicates that thyroidectomy cells do not develop in the same manner as castration cells because of a lack of hormone from the gonads.

Reference has been previously made³ to the experiments published by a number of investigators who have found that the pituitaries of castrated animals contain and secrete an excess of

* Received for publication May 28, 1938.

gonadotropic hormones. If castration cells are the same as thyroidectomy cells one might expect to find a simultaneous secretion of thyrotropic hormone by the castration cells, but in the literature the weight of evidence is against any increased thyroid activity in the castrated animal. Furthermore, the pituitary of the thyroidectomized animal, which contains an abundance of thyrotropic hormone,⁵ contains a decreased amount of gonadotropic hormone in the female, while no data in the literature have been found for the male. Severinghaus⁷ writes: "We are at a loss to explain the failure of glands (pituitary) of either the thyroidectomized or the thyroid treated animals to show increased gonadotropic potency by the implant test. In both instances there is a marked increase in basophilia comparable structurally to the basophilic changes after castration, which do not show increased potency. In the writer's opinion, this discrepancy constitutes a true stumbling block to those who have hopes of correlating structural changes with function, and some satisfactory explanation of the discrepancies must be forthcoming." To me there are no discrepancies and no stumbling blocks if one grants that thyroidectomy cells are not identical with castration cells in structure and produce different secretory products. On the basis of this conception the complete dissociation between formation of thyrotropic hormone and gonadotropic hormone determined by implant tests for potency is satisfactorily explained.

Recently Nelson and Hickman⁸ have opposed some of my conclusions because of the results of their experiments dealing with estrone injections into thyroidectomized rats. They state: "The effectiveness of oestrone in preventing and correcting the changes which occur in the hypophysis following thyroidectomy is similar to its action on the changes that follow castration and appears to be evidence against the idea that the basophiles which react to the 2 operations represent 2 different cell types." Severinghaus⁹ in a review article writes that he and Smelser in some unpublished experiments find that "the injection of 5 r.u. of progynon daily into thyroidectomized female rats prevented vacuolation of pituitary basophiles over 50 days." Previously, however, Hohlweg and Junkmann¹⁰ had found that progynon (estrogenic) injections prevented the development of castration cells but not thyroidectomy cells.

It would seem to me that the conclusions drawn by Nelson and Hickman would hold only if it were proved that estrone had a selective action on the basophils that develop into castration cells and on no other cell. In the dosage they used (40 R.U. daily) no diffuse effects would be anticipated, but in the voluminous literature on the effect of estrone, most workers are agreed that repeated estrone injections in large doses degranulate all granular cells of the pituitary, including acidophils, producing finally a chromophobic pituitary. Hence there is no reason why thyroidectomy cells should not be degranulated, if sufficient dosage is used, even if they represent a different type of cell from that which gives rise to the castration cell.

The following experiments * were carried out in order to gain further evidence of similarity or difference in the reaction of the pituitary after castration and after thyroidectomy to the administration of estrogenic hormone and thyroid extract.

METHODS

White rats from an inbred colony maintained in this laboratory for 5 years were operated upon at 5 weeks of age and killed 5 weeks later. The pituitaries were fixed immediately in Helly's fluid and stained with Mallory's aniline blue collagen stain. The pituitaries of several different rats subjected to different types of experiments were prepared in the same way and mounted on the same slide as a control for staining. Ninety-three rats were studied as follows: 7 males and 6 females gonadectomized and receiving no treatment; 6 males and 6 females thyroidectomized and receiving no treatment; 7 males and 7 females gonadectomized and injected with estrone (theelin in oil, Parke, Davis & Company); 15 males and 13 females thyroidectomized and injected with estrone; 2 males and 2 females thyroidectomized and fed desiccated thyroid extract (U.S.P., Abbott Laboratories) in a dosage known to prevent the development of thyroidectomy cells; 4 males and 3 females gonadectomized and fed thyroid extract in the same or larger dosage; 4 males and 3 females not operated upon and untreated as controls for age and sex; and 5 males and 3 females not operated upon and injected with estrone.

* The results were read in brief before the American Association of Pathologists and Bacteriologists, May 3, 1938, *Am. J. Path.*, 1938, 14, 650-651.

RESULTS

Five weeks after operation the histological differences between castration and thyroidectomy cells are much more obvious than at later periods. The castration cells have not yet developed their signet ring appearance and so contain no hyaline material in a vacuolated space as occurs in later stages. They appear as numerous enlarged and uniformly granulated basophils comprising about 50 per cent of the anterior pituitary, and the acidophils are not noticeably altered. On the other hand, after thyroidectomy the pituitary has attained the maximum change at 5 weeks, the predominating cells being thyroidectomy cells containing large amounts of intracellular hyaline, with very fine, poorly staining granules in the remaining portion of the cytoplasm. Nearly all the acidophils have disappeared.

In varying the dosage of estrone injected so as to determine the dosage effective for suppressing castration cells, in the first experiments a few rats were given an ineffective dosage (see Tables I and II). In a few of the earlier experiments treatment was not begun until 2 or 3 weeks had elapsed after operation and this was found not to be a suitable method. In all other experiments estrone or thyroid extract was administered 6 times a week from the day of the operation to the day of killing the rat. As body growth is inhibited by thyroidectomy, the dosage stated really represents a larger dose per gram of body weight in the case of the thyroidectomized than in the gonadectomized rats, which tend to be heavier than normal.

The microscopic sections were first examined and classified without knowledge of the nature of the experiment. Without exception all pituitaries of thyroidectomized rats were recognized as such whether the rats were untreated or had been injected with estrone. In 6 thyroidectomized males injected with a total of 1200, 1450, 2320, 2900, 2900 and 4350 international units of estrone, thyroidectomy cells were present as the predominating cell. After the largest doses only, were thyroidectomy cells slightly reduced in number. All 5 gonadectomized males injected with 1450, 2320, 2900, 3360 and 4350 international units showed complete suppression of castration cells so that a castration effect was unrecognizable. In 7 thyroidectomized females injected with a total of 595, 615, 615, 1160, 1500, 2900 and 2900 international units,

TABLE I
Data on Female Rats

Number of rats	Operation	Treatment. Total dosage in postoperative period	Period over which treatment administered	Histological appearance of pituitary	
				Normal pituitary	No conspicuous changes
3	None	None	Last 2 weeks	"	"
1	"	240 I.U. estrone	" 3	"	"
1	"	950 "	" 5	"	"
1	"	635 "	"	"	"
6	Gonadectomized	None	Last 2 weeks	Typical castration changes	Very few castration cells
1	"	240 I.U. estrone	" 3	"	"
1	"	950 "	" 5	"	"
1	"	635 "	" 5	"	"
1	"	1160 "	" 5	"	"
1	"	1500 "	" 5	"	"
1	"	2900 "	" 5	"	"
2	"	None	"	Typical thyroidectomy changes	"
6	Thyroidectomized	260 I.U. estrone	Last 2 weeks	"	"
3	"	750 "	" 3	"	"
3	"	595 "	" 5	"	"
1	"	615 "	" 5	"	"
2	"	1160 "	" 5	"	"
1	"	1500 "	" 5	"	"
1	"	2900 "	" 5	"	"
1	"	0.928 gm. thyroid extract	Last 5 weeks	Normal pituitary	"
2	"	1.151 "	" 5	"	"
1	Thyroidectomized	0.957 gm. thyroid extract	Last 5 weeks	Typical castration changes	"
1	"	1.677 "	" 5	"	"
1	Gonadectomized	1.972 "	" 5	"	"
1	"	"	" 5	"	"
1	"	"	" 5	"	"

TABLE II

Data on Male Rats

Number of rats	Operation	Treatment. Total dosage in postoperative period	Period over which treatment administered	Histological appearance of pituitary
4	None	None	..	Normal pituitary
I	"	240 I.U. estrone	Last 2 weeks	No conspicuous changes
I	"	850 "	" 3 "	" "
I	"	900 "	" 3 "	" "
I	"	580 "	" 5 "	" "
I	"	2800 "	" 5 "	" "
7	Gonadectomized	None	..	Typical castration changes
I	"	240 I.U. estrone	Last 2 weeks	" "
I	"	580 "	" 5 "	Fewer castration cells
I	"	1450 "	" 5 "	Castration cells degranulated
I	"	2320 "	" 5 "	No castration effect
I	"	2900 "	" 5 "	" "
I	"	3360 "	" 5 "	" "
I	"	4350 "	" 5 "	" "
6	Thyroidectomized	None	..	Typical thyroidectomy changes
I	"	220 I.U. estrone	Last 2 weeks	" "
2	"	260 "	" 2 "	" "
3	"	750 "	" 3 "	" "
3	"	580 "	" 5 "	" "
I	"	1200 "	" 5 "	" "
I	"	1450 "	" 5 "	" "
I	"	2320 "	" 5 "	" "
2	"	2900 "	" 5 "	" "
I	"	4350 "	" 5 "	" "
I	Thyroidectomized	0.957 gm. thyroid extract	Last 5 weeks	Normal pituitary
I	"	1.638 "	" 5 "	" "
2	Gonadectomized	0.957 gm. thyroid extract	Last 5 weeks	Typical castration changes
I	"	1.639 "	" 5 "	" "
I	"	2.389 "	" 5 "	" "

thyroidectomy cells were present in all pituitaries, though slightly reduced in number with the largest dosage, while all 5 gonadectomized females injected with 635, 1160, 1500, 2900 and 2900 units showed no castration cells and were unrecognizable as pituitaries from castrated animals.

In 4 gonadectomized males fed a total of 0.957, 0.957, 1.639 and 2.389 gm. of thyroid extract, the castration cells were not affected in the slightest, while 2 male thyroidectomized rats fed 0.957 and 1.638 gm. showed no thyroidectomy cells. In 3 gonadectomized females fed 0.957, 1.677 and 1.972 gm. of thyroid extract castration cells were not affected, while 2 thyroidectomized females fed 0.928 and 1.151 gm. of thyroid extract showed complete suppression of thyroidectomy cells. Figures 1 to 4 illustrate typical examples of pituitaries in each category. Tables I and II indicate the number of rats and the dosage used.

In the thyroidectomized rats the effect of estrone was obvious in its characteristic influence on the gonads. After large doses the testes were reduced to minute organs comparable to the effect of hypophysectomy because degranulation of the basophils which produce gonadotropic hormone is caused by estrone, and synchronous with this is the cessation of secretion of gonadotropic hormone, as determined by implantation experiments. In the thyroidectomized rats treated with estrone there was an increase in number of chromophobes, just as occurs in normal rats after estrone administration. This chromophobe increase gave the pituitaries of the treated thyroidectomized rats a slightly different histological pattern from that in the untreated thyroidectomized rats. Complete thyroidectomy was evidenced by marked inhibition of growth of the kidney, the short, plump external appearance of a dwarfed rat, and the characteristic histological appearance and gross enlargement and engorgement of the pituitary. The dosage used in most of these experiments enumerated above was much greater than that used by Nelson and Hickman, although small doses likewise had no effect on the thyroidectomized rats used in the preliminary experiments.

DISCUSSION

In any normal pituitary there are great variations in the histological characteristics of the basophils. The conventional explana-

tion of this is that the different appearances represent different phases of secretory activity. It is not unreasonable or contrary to evidence to assume as a hypothesis that some of these variations of staining and degree of granularity really indicate that there are different varieties of basophilic cells producing different hormones. Granting this as a possibility, it would be conceivable that under normal conditions basophilic cells forming different hormones cannot be distinguished from each other on histological grounds, but that when a peripheral endocrine organ is ablated, and the pituitary cell which affects that peripheral organ reacts to the ablation, then different varieties of basophils can be dissociated. It would be difficult to determine with certainty from what original cell thyroidectomy cells are derived, that is, whether they are formed from a precursor chromophobe, or whether a basophil of a single type can differentiate in different directions to a castration cell and to a thyroidectomy cell. To some histologists the conception of more than one type of basophil seems to be a violation of fundamental principles, but to me it is even more difficult to accept that merely two types of secretory cells (one acidophilic and one basophilic) produce the numerous hormone effects that can be concretely demonstrated. Especially is this so when one realizes that the hormone effects need not occur synchronously but can be dissociated from each other. In any case, whatever may be the origin of the thyroidectomy cells, after they have developed they appear to be a different type of cell from the castration cell, structurally and functionally.

From what we know of the reciprocal relations between the pituitary and the peripheral endocrine organs we would expect the results described above. For instance, the pituitary producing the thyrotropic hormone stimulates the thyroid to secrete thyroid hormone, which in turn acts upon the pituitary, inhibiting its production of thyrotropic hormone, as has been determined by implantation experiments (Hohlweg and Junkmann¹⁰). In consequence of this the thyroid stops secreting its hormone. As a result of this, the inhibiting effect upon the pituitary is removed and it again resumes secretion of the thyrotropic principle. If the thyroid is ablated, the thyroidectomy cell that reacts in the pituitary is presumably the cell in the pituitary that is producing the thyrotropic hormone, and it would be expected that only the pres-

ence of the hormone secreted by the thyroid gland, and not estrone, would prevent the development of this cell and maintain a normal cellular pattern in the pituitary.

Estrone in large doses is well known to have a diffuse effect. It causes degranulation of basophils in both males and females and through this effect alters the structure of the gonads in both sexes. It retards body growth and since this is accompanied by degranulation of the acidophils, it probably produces the effect on growth by lessening the production of growth hormone by the acidophils. Consequently in large doses one would anticipate that estrone would affect the production by the pituitary of thyrotropic hormone and other hormones. Some of the doses used in these experiments were large enough to produce some of these diffuse effects, and yet the thyroidectomy cells were not prevented from developing.

SUMMARY AND CONCLUSIONS

Rats were operated upon at 5 weeks of age and killed 5 weeks later. Forty-four were thyroidectomized and 34 were castrated. In each group some were injected with estrone, some were fed thyroid extract, and some were untreated.

The lack of effect on thyroidectomy cells of estrone injected in a dosage greater than adequate to suppress castration cells, and the failure of castration cells to respond to thyroid extract in doses greater than required to suppress thyroidectomy cells indicate that thyroidectomy cells probably are structurally and functionally different cells from castration cells, whatever may be their derivation.

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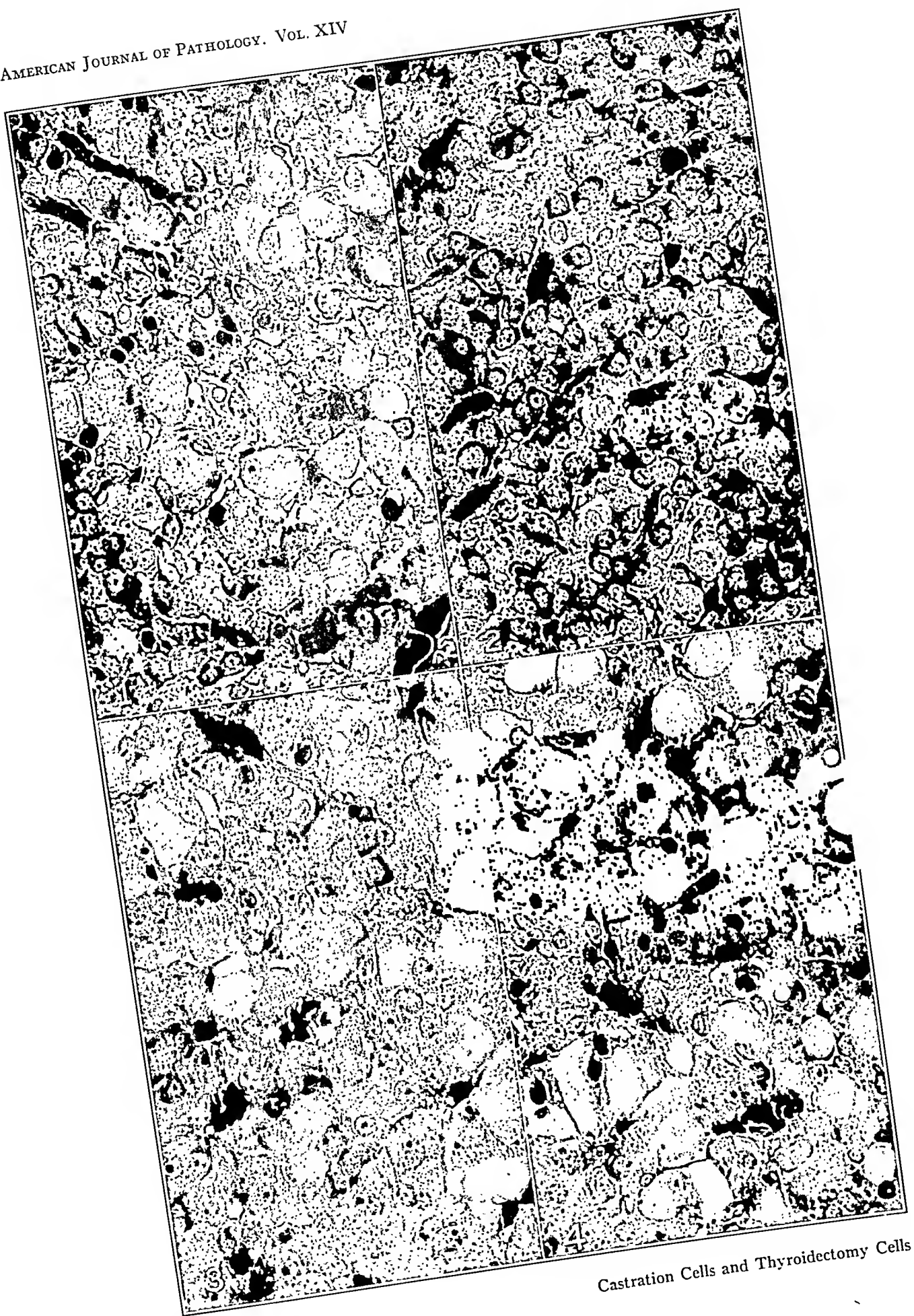
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DESCRIPTION OF PLATE

PLATE 146

- FIG. 1. Male rat (12-S-3) castrated and receiving no treatment. Killed 5 weeks after operation. Typical castration effect, consisting of increased number and size of basophils. Acidophils normal. $\times 523$.
- FIG. 2. Male rat (12-S-1), litter-mate of preceding rat, injected with a total dose of 2320 I. U. estrone for 5 weeks after operation. No castration cells present. $\times 523$.
- FIG. 3. Male rat (12-S-4), litter-mate of preceding rat, thyroidectomized and receiving no treatment. Killed 5 weeks after operation. Typical thyroidectomy effect, consisting of marked loss of acidophils and development of thyroidectomy cells containing intracellular hyaline. $\times 523$.
- FIG. 4. Male rat (12-S-2), litter-mate of preceding rat, thyroidectomized and injected with a total dosage of 2320 I. U. estrone for 5 weeks after operation. Typical thyroidectomy cells abundant and loss of acidophils apparent. $\times 523$.



Castration Cells and Thyroidectomy Cells

HISTOLOGICAL VARIATIONS IN AUTONOMIC GANGLIA AND GANGLION CELLS ASSOCIATED WITH AGE AND DISEASE *

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Histological variations in autonomic ganglia and ganglion cells have been described by a number of investigators. Some have regarded the more marked changes as representing pathological lesions; others have regarded all the observed variations as representing structural changes falling within the normal range of variability. Most of the studies of which the results have been published have been carried out on preparations of ganglia of the sympathetic trunks and prevertebral plexuses obtained at autopsies following death due to widely differing causes and within wide age limits; some have been on preparations of sympathetic ganglia removed in the surgical treatment of certain diseases. The studies carried out on material of the latter type have revealed no specific histopathological changes in the ganglia which could be correlated with the disease in question. The histological variations observed in such material fall into the same general categories as those observed in preparations of ganglia obtained from apparently normal individuals and individuals with other diseases. Certain investigators, notably Craig and Kernohan,¹ consequently have advanced the opinion that most of the histological changes observed in preparations of autonomic ganglia can be explained most satisfactorily on the basis of advancing age.

Some of the histological variations observable in preparations of autonomic ganglia undoubtedly are correlated with the ages of the subjects. Others probably are associated with disease either as causative factors or accompaniments. Regardless of the specific relationships of lesions of the autonomic ganglia or ganglion cells to a disease process with which they are associated, the functional modifications associated with them may play a significant rôle in the progress of the disease and its sequelae. The establishment of norms for human autonomic ganglia and ganglion cells in the

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several age groups, consequently, would be desirable, but, on the basis of our present knowledge; such an undertaking must be regarded as hazardous.

MATERIALS AND METHODS

The present study is based mainly on preparations of ganglia of the sympathetic trunks and the celiac plexus obtained in an extensive series of autopsies following death at ages ranging from 5 weeks to 78 years. The cases have not been selected with reference to disease, but the causes of death vary within a wide range. Preparations of sympathetic ganglia removed in the surgical treatment of disease in approximately 50 patients, ranging in age from 6 to 71 years, also have been available for study.

Most of the material has been fixed in 10 per cent formalin and stained with toluidine blue and erythrosin, hematoxylin and erythrosin, or cresyl violet. The rest has been prepared by various modifications of the Cajal silver technic.

HISTOLOGICAL DATA

Relative Frequency of Ganglion Cell Types: As observed in silver preparations, nearly all the ganglion cells in the autonomic ganglia of children and young adults possess only long dendrites. According to de Castro,² all the autonomic ganglion cells conform to this type during fetal and early postfetal life. In our preparations of ganglia of young adults, ganglion cells characterized by short intracapsular and glomerular dendrites occur only rarely except in the cephalic parasympathetic ganglia, in which short dendrites are common. Those with both short and long dendrites are more abundant. Since ganglion cells of the latter type are more abundant in the ganglia of adults than in children, it must be assumed that short intracapsular dendrites may arise late in the process of differentiation. This opinion has been expressed by de Castro² who designated the short intracapsular processes "secondary dendrites." Preparations of ganglia in the more advanced age groups show both ganglion cells with short and glomerular dendrites and those with both long and short dendrites in greater abundance than in preparations of ganglia of young adults.

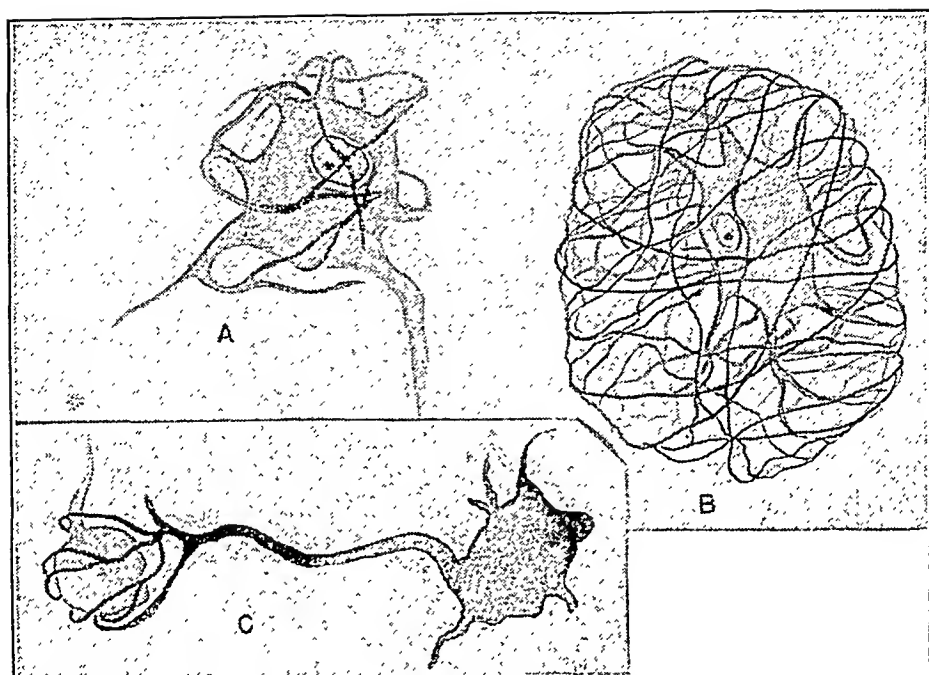
The progressive changes in the dendrites of the ganglion cells are less marked in the cephalic autonomic ganglia than in other

parts of the autonomic system, since short dendrites are relatively abundant in these ganglia in the younger age groups as well as in the more advanced. Intracapsular dendrites are not uncommon in the cephalic autonomic ganglia in all age groups. Many of them are very short and terminate in knob-like enlargements; others are much longer but do not penetrate the ganglion cell capsule. The extracapsular dendrites also are relatively short. Slavich³ has emphasized the preponderance of ganglion cells with short dendrites in the cephalic autonomic ganglia.

Dendritic Modifications: Silver preparations of ganglia in the more advanced age groups show various modifications of the dendrites of some of the ganglion cells. Intracapsular dendrites and arborizations of extracapsular dendrites are more abundant in many of the ganglia in the advanced age groups than in any of the younger ganglia. New dendrites obviously arise relatively late in life. Some investigators, particularly de Castro^{4,5} and Levi,⁶ have supported the assumption that autonomic ganglion cells may undergo continuous growth and differentiation throughout life.

In some of the ganglia in the more advanced age groups (40 years or over) elaborate pericellular dendritic nests are not uncommon. These are formed by dendrites which are wrapped around the ganglion cell bodies from which they arise or the cell bodies of other ganglion cells in the vicinity (Text-Fig. 1). These structures, which de Castro has designated "false articulation nests," probably owe their origin to the enormous elongation of the dendrites involved. In the most elaborate ones the dendritic branches superficially resemble the terminal branches of axons (Text-Fig. 1B and Fig. 4). In the simpler pericellular nests formed on the cell bodies of ganglion cells by the terminal branches of dendrites of adjacent ganglion cells the processes involved retain their typical dendritic appearance (Text-Fig. 1C).

Budding and hypertrophy of the dendrites occur frequently in some of the ganglia in the advanced age groups. Short dendrites not infrequently present a tuberoso or beaded appearance and terminate in club shaped enlargements. Longer dendrites sometimes present irregular local thickenings by virtue of which they appear highly distorted (Text-Fig. 2). In some instances dendrites give rise to new processes of variable length and caliber which form more or less complex brushes and tracts (Figs. 1, 2, 3).



TEXT-FIG. 1. Simple (A) and complex (B) pericellular nests formed by dendrites of same cells, and simple pericellular nest (C) formed by dendrite of adjacent ganglion cell, in celiac ganglion, age 44 years.



TEXT-FIG. 2. Celiac ganglion cells with thickened and distorted dendrites, age 78 years.

Structures of this kind have been reported by de Castro, particularly in cases of tabes, alcoholism and multiple sclerosis. Dendritic glomeruli involving two or more ganglion cells also are not uncommon in most of the autonomic ganglia.

Chromidial Substance: The chromidial substance in the ganglion cells may be studied satisfactorily either in the toluidine blue-erythrosin or the hematoxylin-erythrosin preparations. In most of the ganglia of children and young adults in our series the chromidial substance is distributed more or less uniformly throughout the cytoplasm in the majority of the ganglion cells (Fig. 5A and B). Some cells contain a more abundant supply of chromidial substance than others; consequently they react more strongly to the basic stain. In those with only a meager supply of chromidial substance this material usually is distributed in the peripheral zone of the cytoplasm (Fig. 5C), leaving the perinuclear zone relatively devoid of chromidial bodies. Less frequently the chromidial substance is aggregated in the perinuclear zone (Fig. 5D). These observed variations in the quantity and distribution of the chromidial substance in the ganglion cells probably are associated with different phases in the functional activity of these cells.

In some of the ganglia obtained at autopsy, particularly in the advanced age groups and nearly all of those in the surgical series, the supply of the chromidial substance is relatively meager in the great majority of the ganglion cells. The chromidial substance present in these cells in most instances exists in minute granules or as chromidial dust (Fig. 5E). Most of the ganglion cells which contain but little chromidial substance also exhibit some diminution in the size of the nucleus and in the quantity of intranuclear chromatin. Some of these ganglia also include hyperchromatic ganglion cells. The latter usually exhibit shrinkage both of the nucleus and of the cytoplasm (Fig. 5F).

Pigmentation: Melanotic pigment in the cytoplasm of some of the ganglion cells is a common phenomenon in all the ganglia of individuals 30 years of age or over in our series. Ganglion cells containing some melanotic pigment also occur in some of the ganglia in the younger age groups. The youngest in our series which show ganglion cells with appreciable amounts of melanotic pigment are sympathetic trunk ganglia of a patient 11 years of age with progressive muscular dystrophy. Traces of melanotic

pigment in ganglion cells have been reported in younger material. Some of the ganglia in our series which fall within the age limits of 18 to 25 years show moderate pigmentation of some cells, but none below the age of 35 years show marked pigmentation. Some of the ganglia in the most advanced age groups also show only moderate pigmentation.

The quantity of melanotic pigment in pigmented ganglion cells varies within wide limits. In moderately pigmented cells the pigment granules may be distributed in a narrow peripheral zone or aggregated in a restricted portion of the cell body, usually adjacent to the base of one of the larger dendrites. In occasional cells the pigment appears aggregated in a cap shaped mass at one side of the nucleus. As the pigment increases in amount it replaces more and more of the cytoplasm until the entire cell body outside the nucleus appears filled with this material. In some ganglion cells masses of pigment granules occur also in the dendritic processes. In cases of excessive pigmentation masses of pigment outside the ganglion cells are not uncommon, although some of the ganglion cells remain devoid of pigment (Fig. 6).

Many moderately pigmented ganglion cells exhibit no other histological changes except some reduction in the quantity of chromidial substance. Excessive pigmentation probably always is accompanied by other degenerative changes in the ganglion cells and results in the death of many of these cells. As the normal cytoplasmic constituents are replaced by pigment granules the ganglion cells undoubtedly become functionless. In silver preparations of heavily pigmented ganglia the dendrites and axons of many of the ganglion cells that are most heavily laden with pigment are not impregnated, although the processes of adjacent ganglion cells which are devoid of pigment or only moderately pigmented are impregnated perfectly (Fig. 6). Excessive pigmentation undoubtedly results in necrosis of a large percentage of the ganglion cells. In the most heavily pigmented ganglia in our series the majority of the ganglion cells obviously are necrotic. Even in these ganglia many of the ganglion cells are devoid of pigment. In moderately pigmented ganglia only the most heavily pigmented cells appear to be necrotic.

The most heavily pigmented ganglia in our series are those that have been obtained following death from carcinoma. They fall

within an age range of 46 to 77 years. In general the younger ones are less heavily pigmented than the older but the difference is not very marked except in the most extreme cases. The youngest ganglia in this group are much more heavily pigmented than most of the other ganglia in the same age group and some of those in the most advanced age group. The excessive pigmentation of the autonomic ganglion cells in this group of patients undoubtedly is associated with the malignant disease. Excessive pigmentation of the autonomic ganglion cells probably is a constant accompaniment of carcinoma, particularly in its advanced stages.

Other Cytological Variations: In the ganglia of children and young adults in our series, except those obtained from surgical cases, the ganglion cells appear highly uniform in their internal structure and present no variations that could be regarded as pathological. Preparations of some of the ganglia obtained at autopsy beyond the ages of young adults and those of nearly all the ganglia in the surgical series show changes other than pigmentation and variations in the chromidial substance, in some of the ganglion cells, which obviously represent degenerative processes. These changes include hyalinization of the cytoplasm in ganglion cells with a meager supply of chromidial substance, hydropic enlargement or edema of a small number of ganglion cells, vacuolization of the cytoplasm in some, neurofibrillar changes in some, and destruction of cytoplasm by phagocytic cells in variable numbers (Fig. 7).

Interstitial Tissue: In toluidine blue-erythrosin and hematoxylin-erythrosin preparations of ganglia of young children the connective tissue framework appears relatively meager. The ganglion cell capsules are inconspicuous and the ganglion cells are closely aggregated in groups which are separated from one another by bundles of axons and long dendrites. Cells probably homologous with neuroglia are present among the dendrites as well as in association with the axons. In preparations of the ganglia of young adults the interstitial connective tissue is somewhat more abundant. The ganglion cell capsules are less delicate than in the younger material, but the ganglion cells within groups remain closely aggregated. The groups are separated somewhat more widely by the increasing volume of the dendrites and axons.

Preparations of the ganglia in the more advanced age groups

which exhibit the narrowest ranges of variation in the ganglion cells show a moderate progressive increase in the amount of connective tissue in the framework of the ganglia and slight thickening of many of the ganglion cell capsules. The interstitial tissue, plus the ganglion cell processes, makes up an appreciably greater percentage of the volume of the older than of the younger ganglia.

Some of the ganglia in our series in all the age groups show relatively wide variations in the interstitial tissue. Some of these variations undoubtedly are associated with acute or chronic infections or other inflammatory processes.

Preparations of all the ganglia obtained following death from acute infectious disease show evidence of marked hyperemia of the interstitial tissue and infiltration with wandering cells, including mainly lymphocytes and mononuclear leukocytes. These cells appear in greatest abundance in the perivascular lymphatics, but also occur throughout the interstitial tissue and, in many instances, within the ganglion cell capsules. In some of the ganglia the interstitial connective tissue shows evidence of hyperplasia.

Preparations of ganglia of individuals with chronic infectious disease usually show less hyperemia of the interstitial tissue than those of ganglia obtained from cases of acute infectious disease. Infiltrating cells also are less abundant, but hyperplasia of the interstitial connective tissue is more marked and proliferation of the cells lining the ganglion cell capsules is not uncommon. Hyperplasia of cells other than those of connective tissue origin also takes place, which accounts for a large percentage of the cellular elements present throughout the interstitial tissue. These factors probably are associated with the inflammatory process.

The above account of the changes observed in the interstitial tissue in autonomic ganglia in acute and chronic infectious disease is in general agreement with those of Staemmler⁷ and Mogilnizky.^{8, 9, 10} These investigators regarded the hyperemia, infiltration and hyperplasia of the interstitial tissue, with accompanying changes in ganglion cells, in the autonomic ganglia in infectious disease as related to the disease process, but not as direct factors in the etiology of the disease.

Preparations of most of the ganglia removed surgically in our series show changes in the interstitial tissue comparable with those observed in the ganglia in instances of chronic infectious

disease. Inasmuch as changes such as these afford evidence of chronic inflammation in the ganglion, they may be regarded as accompaniments of the diseases in question.

COMMENT

The histological data set forth above show that preparations of autonomic ganglia falling within any given age group exhibit certain variations common to all the ganglia in that group, but the ganglia of certain individuals in every age group exhibit a wider range of variation than those of others. This is due in part to the presence of certain variations in some cases which are not common to all in the same age group and in part to the existence in exaggerated form of certain of the common variations. The ganglia in every age group which exhibit only those variations that are common to all ganglia within that group undoubtedly may be regarded as most nearly normal. The variations they exhibit, consequently, are related to age. Variations that exist in some of the ganglia in a given age group and not in others obviously depend on factors other than age. Some of these variations undoubtedly are pathological in some degree. The existence in exaggerated form of certain variations common to all ganglia in the same age group probably is causally related to pathological lesions in the body which either affect the entire organism or at least result in modifications of metabolic functions.

Changes in the autonomic ganglia indicated by the variations in the successive age groups which, according to the criteria suggested above, may be regarded as related to age include the following: growth and differentiation of the ganglion cells from birth to maturity; development of secondary dendrites and other dendritic modifications in some of the ganglion cells during adult life; deposition of melanotic pigment in moderate amounts in some of the ganglion cells, particularly after the age of 30 to 35 years; exhaustion of the chromidial substance in some ganglion cells; degenerative changes in occasional ganglion cells particularly in advanced age, including hydropic enlargement of the cell body, vacuolization or hyalinization of the cytoplasm, neuronophagia in moderate degree, and necrosis; moderate progressive increase in the quantity of interstitial connective tissue from birth to advanced age; and thickening of the ganglion cell capsules in some

degree and the occasional existence of free cells within the ganglion cell capsules, particularly in advanced age. Changes which, according to the same criteria, may be regarded as pathological include the following: elaborate development of dendritic nests, dendritic brushes, and so on, and excessive budding and hypertrophy of dendrites; marked chromidial changes in large numbers of ganglion cells, including diminution of the supply of this substance in some cells and hyperchromatism in others; excessive deposition of melanotic pigment in the ganglion cells; marked degenerative changes in considerable numbers of ganglion cells, particularly in the less advanced age groups, including hydropic enlargement of the cell body, vacuolization or hyalinization of the cytoplasm, neuronophagia and necrosis; hyperemia and infiltration of the interstitial tissues and hyperplasia of both connective tissue and non-connective tissue elements; and marked thickening of ganglion cell capsules with proliferation of the cells lining them.

Histological variations in autonomic ganglia which obviously are related to the age of the individual probably depend on progressive changes in the metabolic processes in the organism. Those that are related to disease undoubtedly depend on factors associated with the disease processes in question. The common occurrence of hyperemia and infiltration of the interstitial tissue in the ganglia in acute or chronic infectious disease, and other conditions in which lesions of ganglion cells are common, strongly suggests that acute or chronic inflammation in the ganglia bears a causal relation to the ganglion cell lesions in many cases. The ganglion cell lesions in such cases, consequently, must be regarded not as causes but as accompaniments of the disease in question. Certain ganglion cell lesions, *e.g.*, excessive pigmentation, probably result from functional depression of the ganglion cells (Dolley and Guthrie¹¹). Since excessive pigmentation is a constant accompaniment of certain pathological states, *e.g.*, arsenic poisoning, cachexia and senile atrophy, it probably must be regarded as a direct result of the pathological state in these cases.

All the observed histological variations in autonomic ganglia that have been regarded as related to disease fall into relatively few general categories. Those associated with diverse diseases, furthermore, may be essentially similar; consequently they cannot be regarded as specifically related to the disease in ques-

tion in any given case. This point of view has been supported by nearly all investigators who have studied the histological variations in autonomic ganglia in relation to disease. As the author¹² has previously pointed out, however, most of the variations observed in autonomic ganglia which obviously are related to disease are indicative of hyperactivity of the ganglion cells. Therefore, it seems not improbable that the autonomic dysfunction associated with these changes may have played a rôle in the disease process in question, particularly in cases in which vasoconstriction was a factor in the disease. The autonomic dysfunction resulting from necrosis or physiological depression of a large percentage of the ganglion cells in certain cases probably also plays a rôle in the disease process in these cases.

SUMMARY

Histological variations in autonomic ganglia and ganglion cells have been studied in preparations of ganglia obtained at autopsy in an extensive series of unselected cases and ganglia removed in the surgical treatment of disease in approximately 50 cases. These ganglia represent an age range from early childhood to senility. The ganglia in every age group that exhibit only variations common to all the ganglia within that group have been regarded as most nearly normal. The variations observed in them, consequently, have been regarded as related to age. Variations not common to all the ganglia within a given age group and certain variations common to all the ganglia in the same age group but existing in exaggerated form have been regarded as pathological in some degree and causally associated with pathological lesions in the body.

The variations that have been regarded as related to age include all changes resulting from normal growth and differentiation both in the ganglion cells and in the interstitial tissue, changes in the chromidial content of ganglion cells associated with normal functional activity, deposition of melanotic pigment in moderate amounts in some ganglion cells, and degenerative changes in occasional cells, particularly in advanced age. Those that have been regarded as related to disease or pathological lesion include the following: marked chromidial changes of ganglion cells; excessive deposition of

in ganglion cells; marked degenerative changes in considerable numbers of ganglion cells, such as hydropic enlargement of the cell body, vacuolization or hyalinization of the cytoplasm, neuronophagia and necrosis; hyperemia and infiltration of the interstitial tissue and hyperplasia of both connective tissue and non-connective tissue elements; and marked thickening of ganglion cell capsules and proliferation of the cells lining them.

The observed variations in the ganglia that seem to be related to disease fall into a few general categories. Those associated with diverse diseases, furthermore, may be essentially similar; consequently, they cannot be regarded as specifically related to the disease in question in any given case.

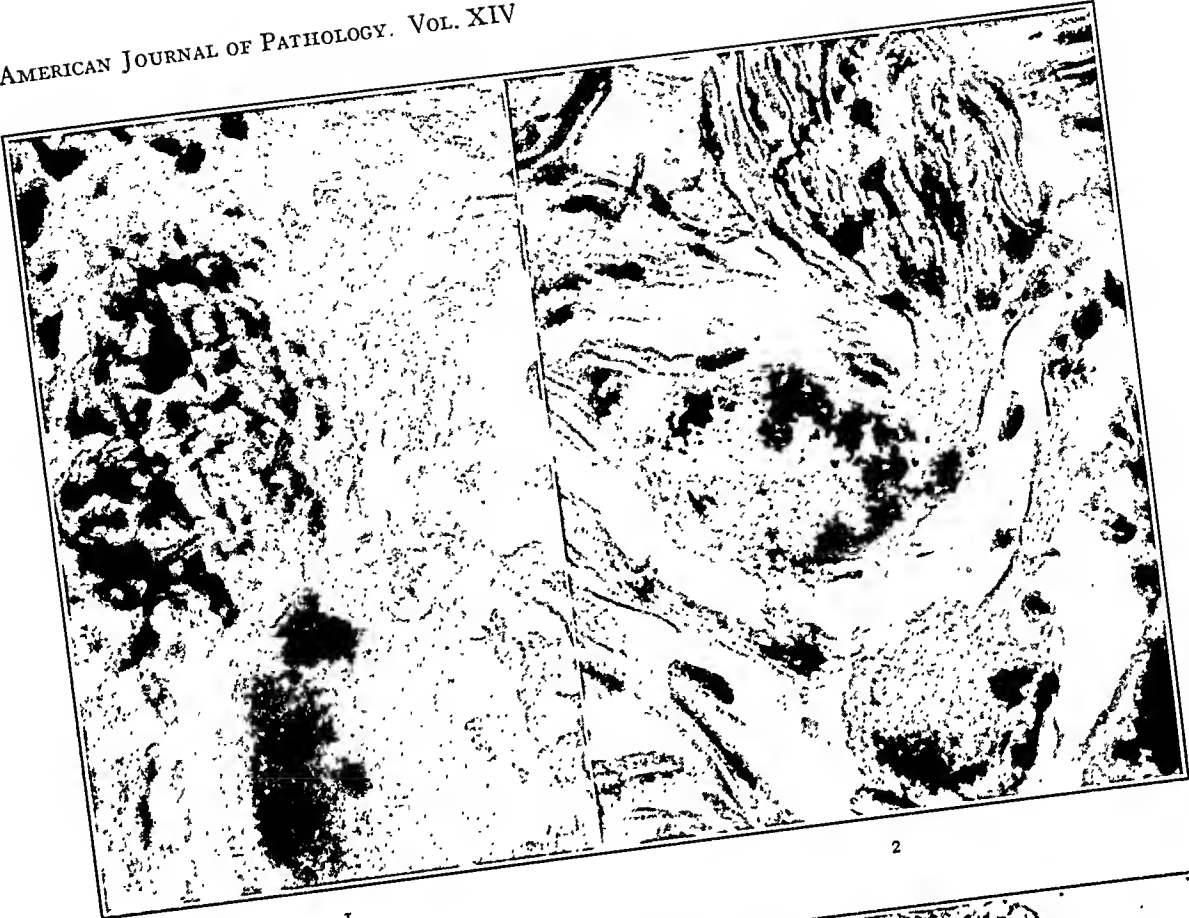
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DESCRIPTION OF PLATES

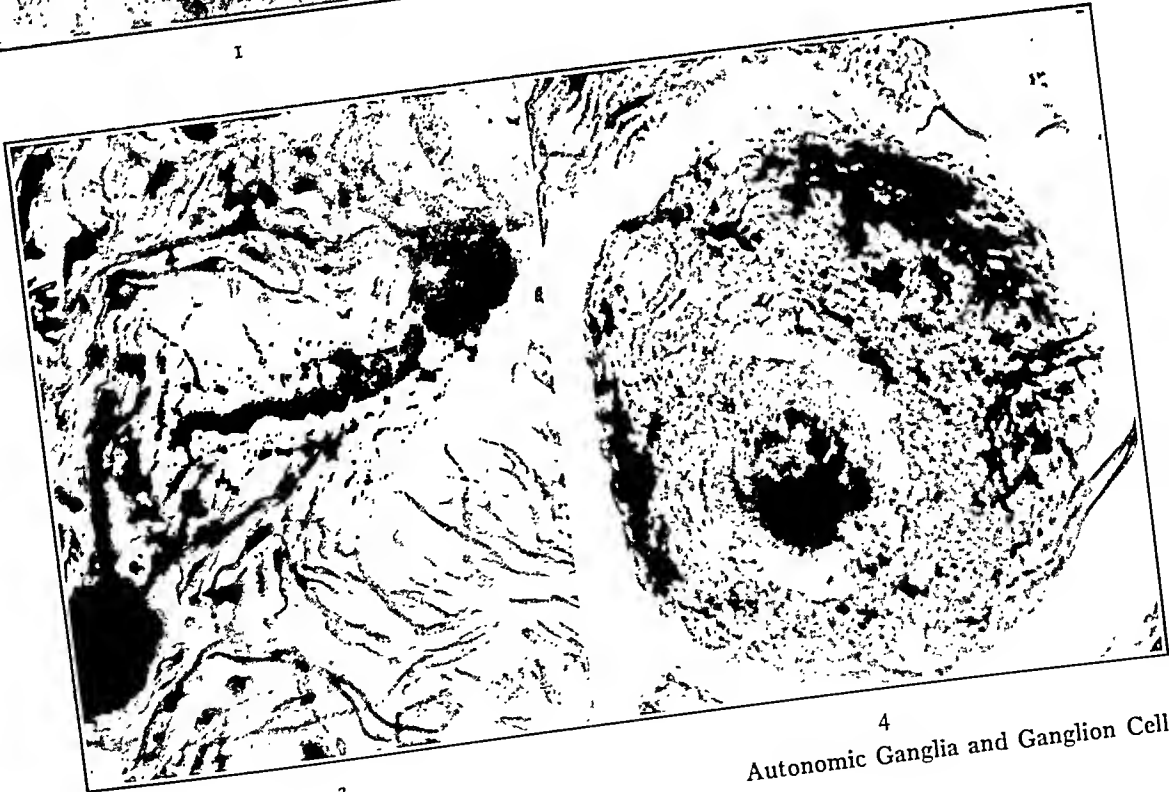
PLATE 147

FIGS. 1, 2, 3 and 4. Ganglion cells showing pathological dendritic modifications from a patient aged 78 years.



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Autonomic Ganglia and Ganglion Cells

PLATE 148

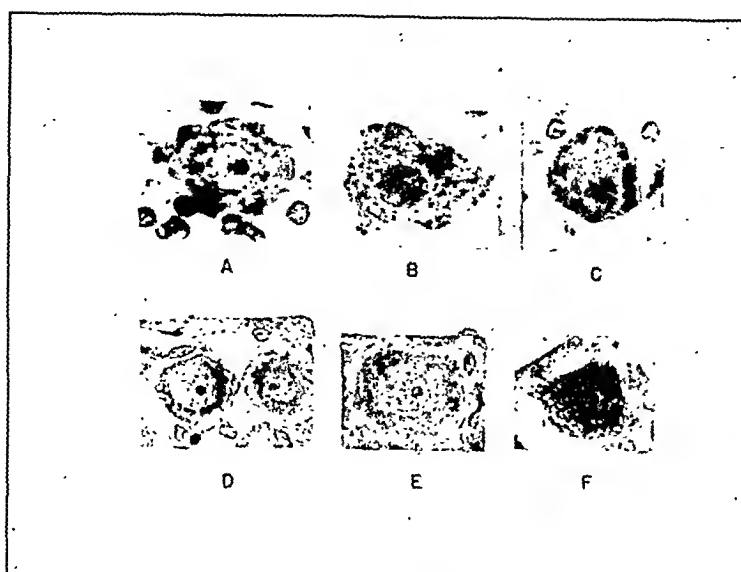
FIG. 5. Autonomic ganglion cells illustrating variations in distribution and quantity of chromidial substance.

A = uniform distribution, chromidial bodies large; B = uniform distribution, chromidial bodies small; C = peripheral distribution; D = perinuclear distribution; E = chromidial dust; F = hyperchromatic cell.

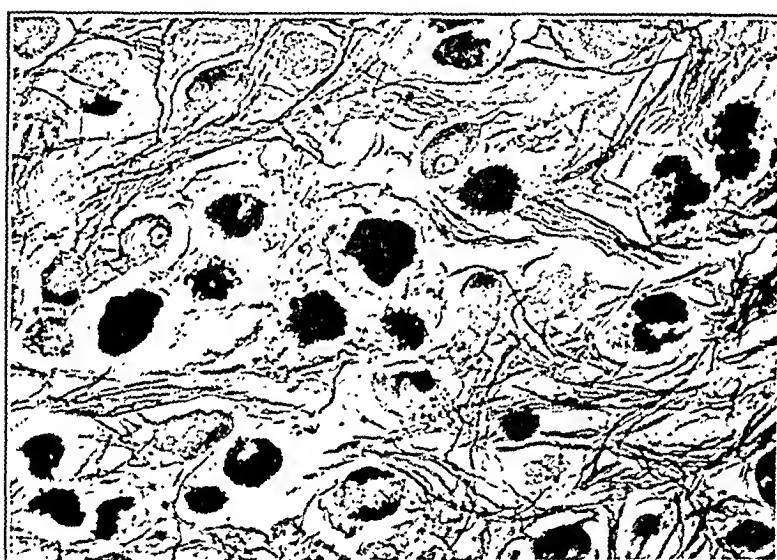
FIG. 6. Heavy pigmentation in sympathetic trunk, patient with carcinoma, age 77 years.

FIG. 7. Ganglion cells.

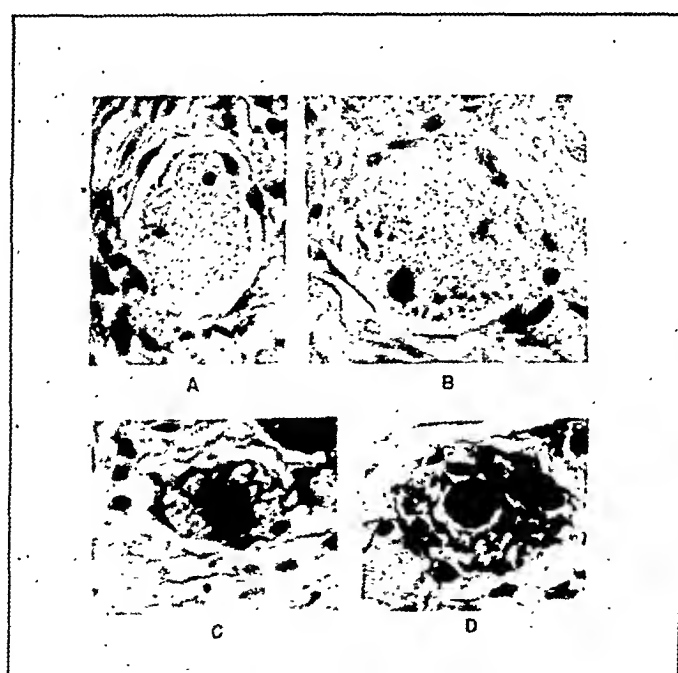
A = hyaline degeneration; B = hydropic enlargement; C = vacuolization; D = neuronophagia.



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7

MULTIPLE TUMORS OF THE SYMPATHETIC NERVOUS SYSTEM *

REPORT OF A CASE SHOWING A DISTINCT GANGLIONEUROMA, A NEUROBLASTOMA AND A CYSTIC CALCIFYING GANGLIONEUROBLASTOMA

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The sympathetic nervous system is the site of an uncommon but interesting group of tumors — the ganglioneuroma, the neuroblastoma and the paraganglioma. While a few of these tumors are reported as arising from the central nervous system, the great majority arise from the embryonic formative nerve cells of the sympathetic nervous system whose differentiation determines the structure and behavior of the tumor. The malignant type is composed of undifferentiated neurocytes and is usually referred to as the sympathetic neuroblastoma. The benign types may be made up either of ganglion cells and nerve fibers forming the ganglioneuroma or of chromaffin cells producing a paraganglioma. Various transitions between these different forms have been reported^{32, 33, 12} and two or even three types may be included in one tumor.^{16, 54, 23} Moreover, a multicentric origin of these tumors in various parts of the sympathetic nervous system has been noted.^{30, 28} Thus far they are usually of the same type, but undoubtedly they may include all three types occurring independently of each other. Such multicentric tumors would imply a widespread neoplastic change in the sympathetic nervous system.

Usually a single primary ganglioneuroma is described and several excellent reviews of these cases are available^{33, 32, 12} so that a discussion of the literature on these types would be superfluous. However, the occurrence of multiple primary tumors arising from the sympathetic nervous system is not so generally recognized.³³ All three types, but especially the ganglioneuromas and paragangliomas, have been reported as multicentric in origin.

* Received for publication May 9, 1938.

Eight cases of undoubted multiple ganglioneuromas were noted in the literature. The first case, described by Knobellauch in 1843 and quoted by McFarland and Sappington,³³ showed a tumor of nerve cells and fibers in the facial region and another of the same type in the sacral area. Busse¹⁰ described a patient with a ganglioneuromatous mass in the pelvis and another under the ribs. Knauss,²⁸ Kredel and Beneke,³⁰ and Montgomery and O'Leary⁴⁰ described multiple cutaneous and subcutaneous nodules containing ganglion cells and nerve fibers somewhat resembling the neurofibromas of the central nervous system in von Recklinghausen's disease but having their origin in the sympathetic nerve twigs about the cutaneous vessels. Roman and Arnold⁴⁸ describe a peculiar diffuse ganglioneuromatosis due to a widespread embryological disturbance of the sympathetic trunk producing an extensive retroperitoneal growth. Haven and Weil²¹ reported multiple growths in the cervical region with another in the pelvis. Bigler and Hoyne⁶ noted such a tumor in the mediastinum and another under the right clavicle. There is some question as to whether the so-called malignant ganglioneuroma³⁸ is not in reality a multiple tumor belonging to this group.⁵²

The paraganglioma is frequently reported as being bilateral.⁴ Marchetti³⁴ was the first to describe bilateral adrenal tumors and Belt⁴ has noted 7 additional cases. Popken⁴² and Kremer³¹ each describe bilateral paragangliomas of the adrenals. Rosenthal and Willis⁴⁹ note such a bilateral involvement associated with multiple neurofibromas. The work of Huebschmann²⁴ and Masson,³⁵ supported by the reports on carcinoids by Cooke,¹¹ Raiford,⁴³ Reid,⁴⁴ Forbus,¹⁸ and Lewis and Geschickter,³² would indicate the identity of the paraganglioma and the carcinoid (argentaffin) tumors of the intestinal tract. This theory is based on the similarity in the structure and the affinity for chrome salts and silver salts of both these groups of tumors. Accordingly, multiple primary carcinomas of the intestines, such as described by Bunting,⁹ represent multiple malignant paragangliomas of the sympathetic system. These carcinoids tend to be single and benign when they arise in the appendix,⁵³ but multiple and malignant when occurring in the intestines. If subsequent studies confirm the identity of the carcinoid and the paraganglioma, the most frequent multiple tumor of the sympathetic system would be the carcinoid of the intestinal

tract. For instance, Cooke analyzes 104 cases with 8 multiple tumors in the malignant forms and 21 multiple tumors in the benign group.

The neuroblastoma may also be multiple¹⁵ although this has been more difficult to demonstrate because of the ever present possibility of early metastases, such as illustrated in Berner's case.⁵ The combination of a benign type with an independent malignant form arising from the sympathetic trunk probably may occur but no such case has been found in studying the literature. Such an unique case comprising an independent benign and a malignant growth, and a third independent growth intermediate in structure forms the basis of this report.

REPORT OF CASE

Clinical History: The patient was a negro, aged 28 years. He had had "rheumatism" for over a year and had become bedfast. He complained of headache and tender spots on the skull. Following a chiropractic adjustment he developed paralysis from the waist downward, associated with urinary retention and fecal incontinence. X-ray examination of the lower spine and pelvic bones showed "extensive destruction of the entire right ilium with the exception of the crest. This destruction consists of a mottled, moth-eaten appearance characteristic of metastatic malignancy." There was also involvement of the acetabulum and superior ramus of the pubic bones on the right. A similar infiltration was seen in the sacrum and left ilium and lower lumbar vertebrae with collapse of the second and fourth lumbar vertebrae. The X-ray also showed a partly calcified tumor mass high in the pelvis on the left. A few days prior to death symptoms of intestinal obstruction supervened and it was regarded as the immediate cause of death.

POSTMORTEM EXAMINATION

The autopsy showed an intestinal intussusception in the lower ileum. When this was opened two pedunculated polypoid masses were present in the thickened and edematous mucosa. Three independent tumor masses were found in the retroperitoneal region of the pelvis. One was a small oval tumor mass (Figs. 1 and 2b) weighing 48 gm., situated in the right iliac fossa adherent to the right iliac artery and vein and associated with the fascia of the psoas muscle. This mass measured 6 by 5 by 2 cm. It was soft and well outlined and cut easily. The freshly cut surface was granular and friable in appearance and of a dark reddish purple color. At the upper part of this mass there were a few fused

enlarged lymph nodes, some of which showed on cut section considerable blood pigment and granular, friable cellular foci indicating tumor metastases.

The second pelvic mass (Figs. 1 and 2a) was attached to the sigmoid colon and to the lower right brim of the pelvis, weighed 60 gm. and measured 6 by 5 by 4 cm. It was rounded in shape and well outlined. It was firm and rubbery in consistence, cut with some resistance, and the cut surface had a wet, glistening white appearance. Nerves could be traced into and were lost in this mass. In some areas the cut surface showed a loose, moist edematous stroma, while in other places it had a wet, glistening, white homogeneous appearance.

The third mass (Figs. 1 and 2c) was also rounded in shape, weighed 98 gm., and measured 7 by 7 by 5 cm. Its surface was roughened and nodular. It was entirely independent of the other two masses and was buried below the second mass deep in the iliac fossa, being adherent to the endopelvic fascia and to the inner wing of the left ilium (Fig. 2c). There were a few loose blood clots on the outer surface. The mass was mainly cystic in character, enclosing blood clots and hemorrhagic jelly-like material. Its wall was largely calcified and measured 3 to 5 mm. in thickness. Its inner surface was ragged and hemorrhagic. Scattered gray cellular masses were embedded in the wall enclosed by heavy white fibrous tissue. Some of these cellular foci were 1 to 2 cm. in diameter, were soft and friable, and in one field presented a gritty consistence as if composed of sand in soft tissue, suggesting many small calcareous deposits. Nerve fibers could be traced into the wall of this hemorrhagic cyst. A few portions of this cystic wall had a moist, wet, homogeneous white appearance such as occurred in the second mass.

No metastases could be detected in the liver or in any other viscera except in a few large lymph nodes adjacent to the first mass. The bodies of the lumbar vertebrae were softened and crushed, and could be cut easily with the knife. The cut surface had the same reddish gray friable appearance as the cut section of the metastases in the lymph nodes in the first mass. Similar softened grayish tissue extended into the sacrum and the adjacent ileum. No other tumor tissue could be found.

HISTOLOGICAL EXAMINATION

Microscopic examination of the polypoid masses in the small intestine at the site of intussusception showed a diffuse acute inflammatory reaction, in fact in many places a phlegmonous appearance was present not only in the polyp but also in the adjacent intestinal submucosa.

The sections through the mass in the right iliac fossa showed a loose cellular tumor tissue in which there were numerous small cells with scanty cytoplasm and rounded nuclei rich in chromatin embedded in a delicate fibrillar reticulum which often had an intimate relation to the cytoplasm of the cells, many of which were arranged in balls, clumps and clusters with a central mesh of fibrils forming "rosettes" (Figs. 3, 4, 5). The outlines of the tumor cells were often obscure, the cytoplasm scanty and often present at one side of the cell with a tendency to extend into a protoplasmic process that blended with the fibrillated stroma (Fig. 3).

The large lymph nodes adherent to this mass were largely replaced by masses of these tumor cells. Most of the lymphoid tissue not involved by the tumor tissue contained considerable yellowish brown pigment enclosed in masses of large mononuclear phagocytes. This pigment gave a positive Perles' test and was most probably blood pigment. Blocks of the softened vertebrae were cut and lost, but since the gross material was the same as that of the mass in the right iliac fossa and adjacent lymph nodes, it was considered probable that this was the same type of tissue. This cellular tissue showed numerous hemorrhages but there was no intimate relation between the tumor cells and the vessels.

The histological study of the second tumor mass, attached to the sigmoid colon, presented a very different picture. It was composed mostly of loose edematous fibrillar tissue at one side of which was much denser fibrous tissue. In this denser area were larger and smaller nests of ganglion cells (Fig. 11) and interlacing nerve fibers mostly of the non-medullated type. Secondary edema and even cystic degeneration were encountered, especially in the nests of ganglion cells (Fig. 9). Occasionally a few clumps of monocytes somewhat resembling the undifferentiated tumor cells were found on careful search. Some of these were found in the nest of ganglion cells. No evidence of regeneration could be seen

in this tissue, in fact the ganglion cells tended to be degenerated. Silver stains showed typical axis cylinders passing out of the ganglion cells and becoming lost in the surrounding mesh of fibrils. Delicate neurofibrils often suggesting a basket-like mesh could be recognized about some of the ganglion cells. Some of the ganglion cells which tended to be oval or elongated in shape contained a large nucleus with an unusually large distinct nucleolus.

The microscopic appearance of the third cystic mass was quite variable in different fields. In a large part of the tumor the wall of the cyst was composed of dense collagenous fibrous tissue showing considerable tendency to calcification and in some places even osseous metaplasia with pseudo bone marrow formation. In other fields the wall was more fibrillar and scattered ganglion cells could be recognized (Fig. 8). In still other nodular areas of softened tissue, embedded in the cystic wall, hemorrhagic cellular foci occurred with the same clumping of the small cells with rosette formation noted in the first mass described, indicating the presence of neurocytes embedded in a fibrillar syncytial reticulum (Figs. 6 and 7). In other fields the cells were more differentiated and the fibrils were arranged in parallel bundles with tumor cells at each end, giving the appearance of sheaves of wheat. In these areas a few, large, more mature cells were seen suggesting immature ganglion cells (Fig. 8). In fact all transitions from neurocytes and ganglion cells could be identified. In some fields this cellular tissue showed extensive deposits of calcium salts in the fibrils (Fig. 6), thus accounting for the gritty sensation noted in gross.

Anatomical Diagnoses: Ganglioneuroma of sigmoid colon and pelvis; neuroblastoma of pelvis with metastases to regional lymph nodes and widespread skeletal metastases; blood pigmentation of lymph nodes; ganglioneuroblastoma of pelvis with extensive secondary hemorrhage, cystic degeneration, calcification and osseous metaplasia; and acute polypoid phlegmonous enteritis with intussusception and intestinal obstruction.

DISCUSSION

The three tumor masses just described bear no direct anatomical relation to each other. They are derived independently from the lower end of the sympathetic trunk and represent different degrees

of differentiation of the same primitive formative neurocyte with the malignant neuroblastoma with lymph node and skeletal metastases on the one hand and a benign ganglioneuroma on the other. While there may be some question as to the independence of the calcified cystic mass and the more cellular (neuroblastoma) mass on the right, there can be no doubt of the independence of the ganglioneuroma. The evidence that the other two are also independent is based on the failure to find any connection between them grossly and the difference in their microscopic structure. One is a rapidly growing cellular growth with invasion into adjacent lymph nodes and bone, while the other shows extensive hemorrhage, calcification, ossification and all stages in differentiation to adult nerve tissue. This lesion seems to be a stage intermediate between the malignant neuroblastoma and the benign ganglioneuroma, that is, a so-called ganglioneuroblastoma.

These three tumor masses comprise a remarkable instance of three independent primary tumors arising from the lower end of the sympathetic system, each different in structure and appearance and representing three stages in the differentiation of the malignant neuroblastoma to the ganglioneuroblastoma and to the ganglioneuroma. The cystic and hemorrhagic mass with a partly calcified wall contained structures resembling the embryonic neurocytes and also had more differentiated nerve cells and fibers in addition to unusual degenerative changes, especially hemorrhage, cystic degeneration and calcification. No paraganglionic tissue was found but there is no reason why a tumor containing this type may not occur.

The striking tendency to hemorrhage, cystic degeneration and calcification is worthy of additional comment. Edema and cystic degeneration are frequently noted in ganglioneuromas.^{5, 29, 26}

Hemorrhage and cystic degeneration are also commonly noted in malignant neuroblastoma^{2, 45} and ganglioneuroblastoma,¹⁶ but calcification in the cystic wall is unique in the literature, especially in association with osseous metaplasia and bone marrow formation, though McFarland³³ and Berner⁵ do refer to the occurrence of liquefaction and calcification. Cystic degeneration and hemorrhage are also frequently described³² in the paraganglioma.¹⁹

The age at which these tumors occur may be worthy of comment. For many years the malignant neuroblastoma was thought

to be present in young children only and not until 10 years ago were any cases reported in older children, but while they are most common in early life a considerable number of cases in later adult life have been reported. Alyea,¹ for instance, reported a case of a man 55 years of age, and noted two other adults in his series, so that the fact that our case was in an adult is not unusual.

SUMMARY

A case of multiple tumors of the sympathetic nervous system is reported illustrating three types of growth arising from the sympathetic system and representing three stages in differentiation of the formative neurocyte, *viz.* neuroblastoma, ganglioneuroblastoma and ganglioneuroma.

Multiple neurogenous tumors of all types have been reported arising from the sympathetic system. If the theory that the paraganglioma is identical with the carcinoid of the gastro-intestinal tract is true, this is the most common multiple and even single tumor of the sympathetic nervous system.

These tumors often show a tendency to cystic degeneration, hemorrhage and calcification, especially the more malignant types.

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DESCRIPTION OF PLATES

PLATE 149

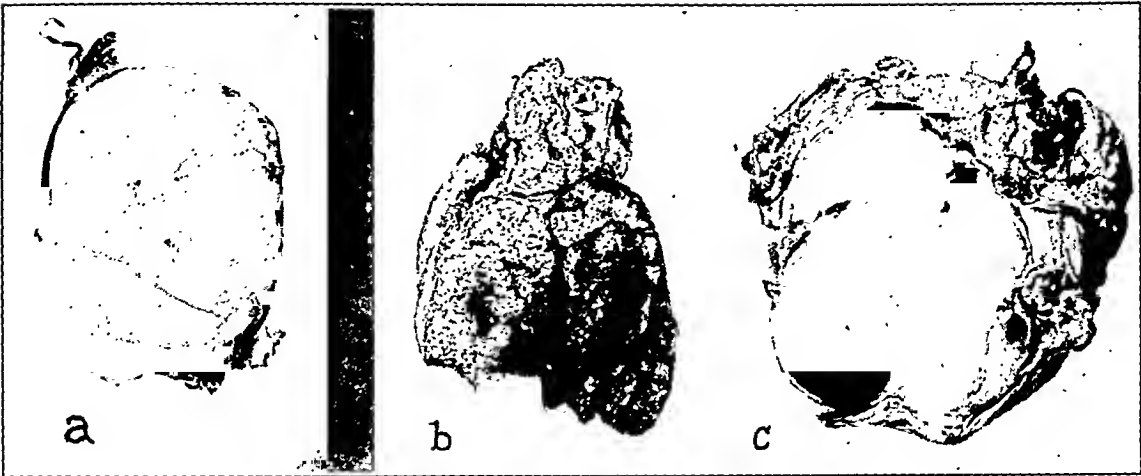
FIG. 1. Gross photographs of cut sections through each of the tumor masses.
2/3 actual size.

a = ganglioneuroma.

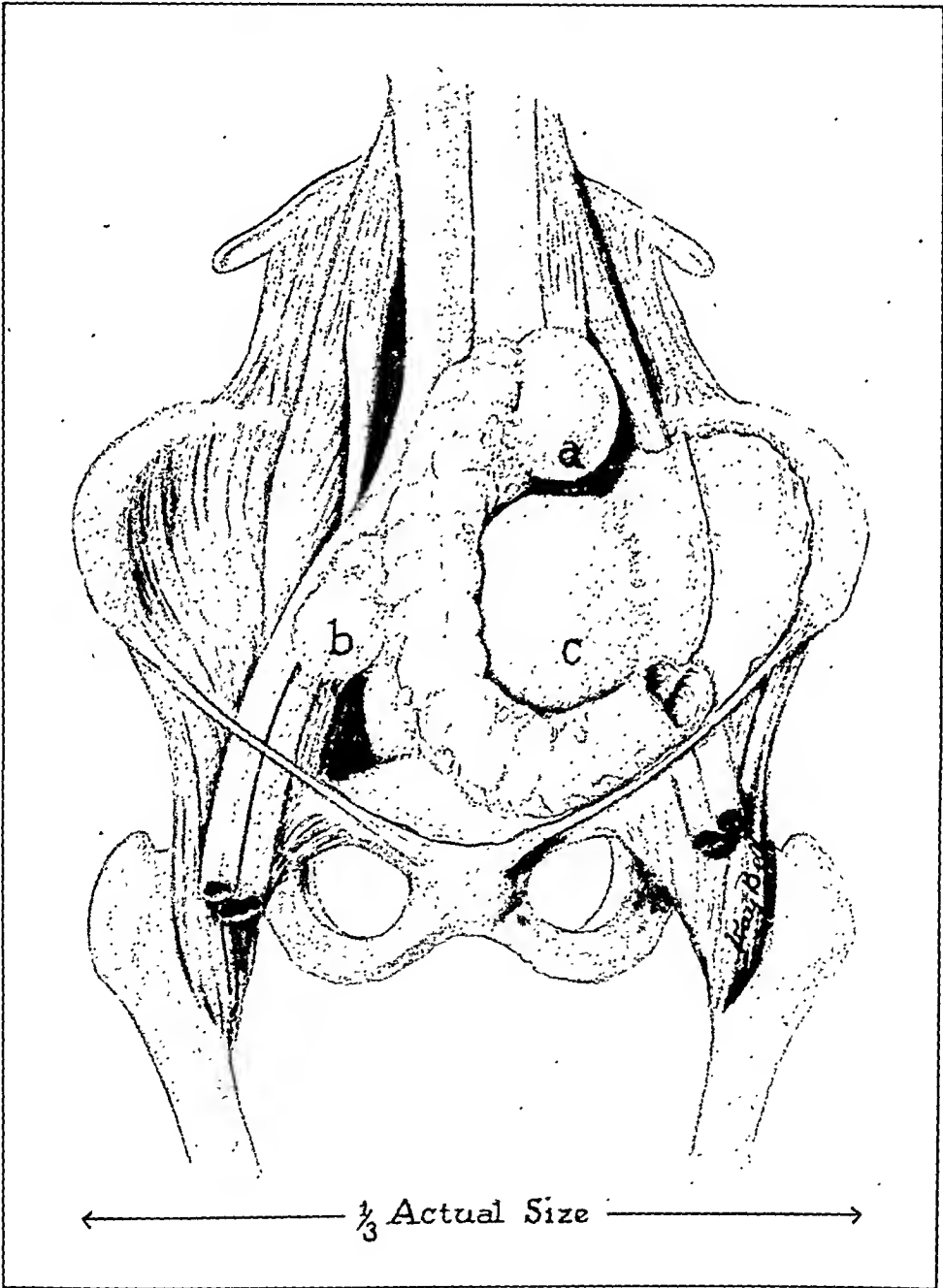
b = neuroblastoma.

c = ganglioneuroblastoma.

FIG. 2. Diagrammatic sketch indicating the location and origin of the tumors.
Lettering as above.



1



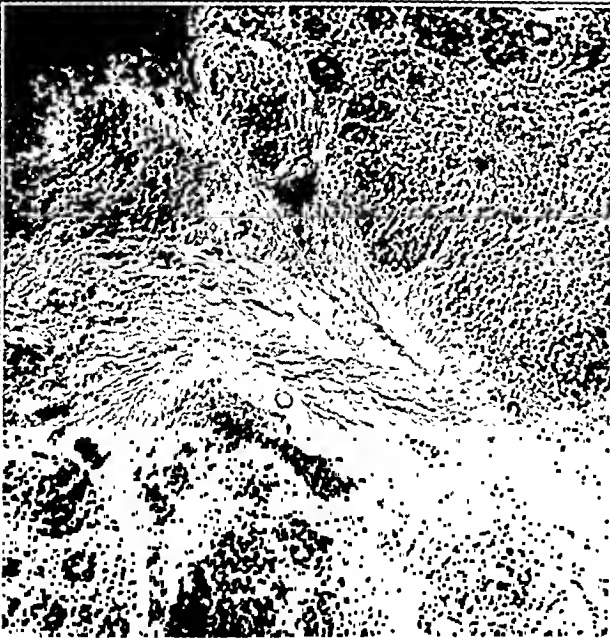
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PLATE 150

FIGS. 3, 4 and 5. Microphotographs of the neuroblastoma (Figs. 1 and 2b) showing rosettes, neurocytes and fibrillar matrix. Hematoxylin and eosin stain. $\times 100$.

FIGS. 6 and 7. Microphotographs of the ganglioneuroblastoma (Figs. 1 and 2c) showing rosettes, fibrillar matrix, hemorrhages (x) and calcification (y). Hematoxylin and eosin stain. $\times 100$.

FIG. 8. Microphotograph of the ganglioneuroblastoma stained by Bielschowsky's method showing immature ganglion cells as well as neurocytes, and at the lower edge masses of neurofibrils. $\times 100$.



3



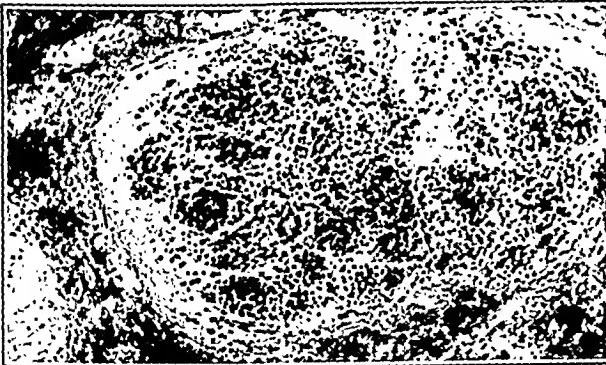
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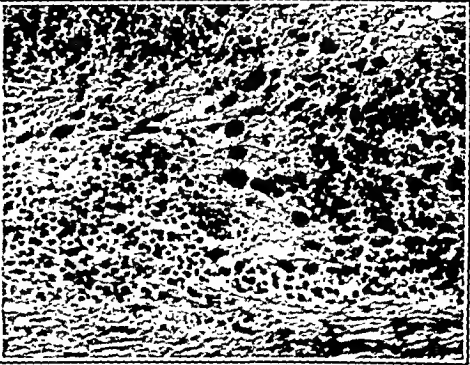
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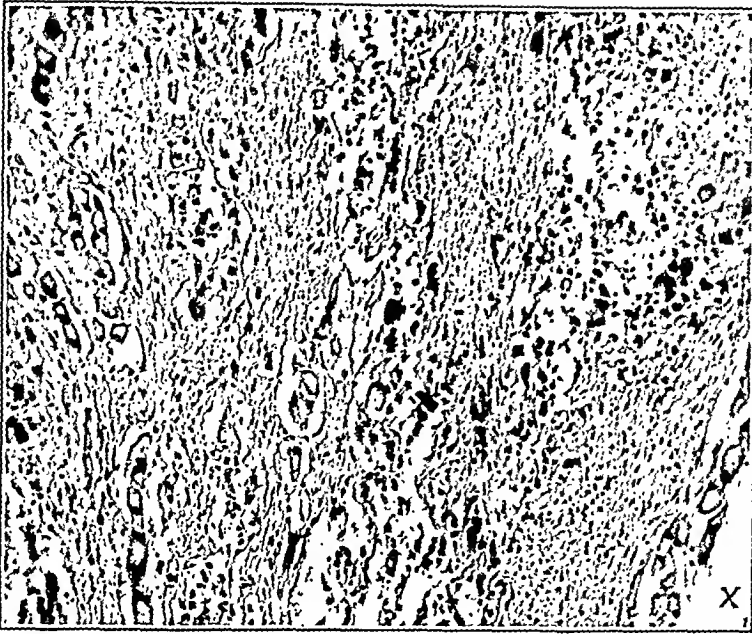
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PLATE 151

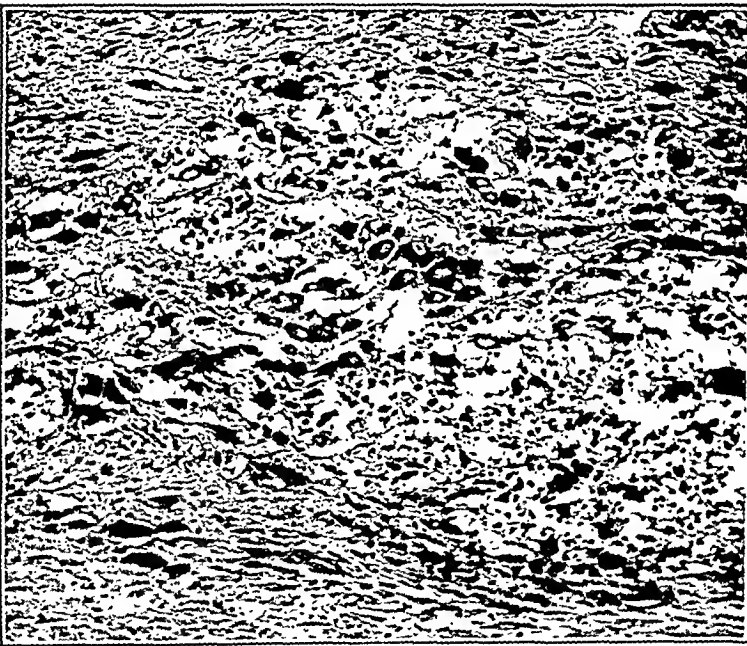
FIG. 9. Microphotograph of a Bielschowsky's silver impregnation preparation of the ganglioneuroma showing clumps of ganglion cells embedded in a neurofibrillar matrix. Note the edema and cystic degeneration, especially at "x." $\times 100$.

FIG. 10. Microphotograph of a Bielschowsky's silver impregnation method preparation of the ganglioneuroma toned with gold chloride. Ganglion cells and neurofibrils are evident. $\times 100$.

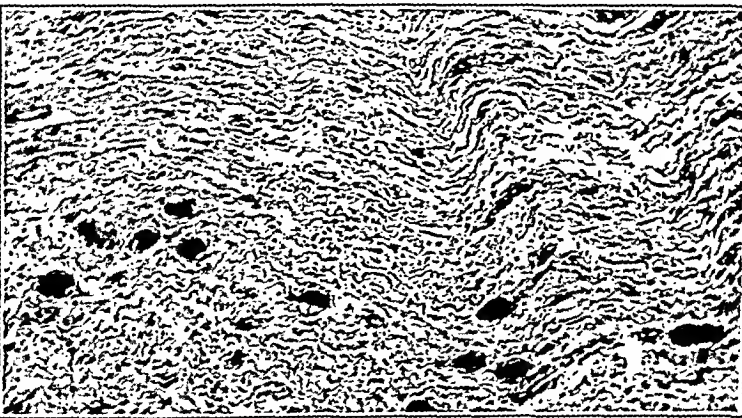
FIG. 11. Microphotograph of a hematoxylin and eosin preparation of the ganglioneuroma. Ganglion cells are embedded in dense bundles of neurofibrils. $\times 100$.



9



10



11

THE AMOUNT OF SPLENIC LYMPHATIC TISSUE AT DIFFERENT AGES *

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In connection with a study of the postmortem weight of the human spleen at different ages (Krumbhaar and Lippincott¹), it was desired to know also the amount of lymphatic tissue in the human spleen at these ages. This question has interested investigators ever since Kölliker² in 1849 established the fact that the malpighian follicles were composed of lymphatic tissue. Among early estimates are von Hessling's³ (1842) that the lymphatic tissue occupied about one-fifth to one-sixth the total volume of the spleen; Gray's⁴ (1854) of from one-eighth to one-fourth; and Kölliker's that it constituted one-fifth to one-sixth the volume of the red pulp. More recently Groll⁵ in 1919 found in healthy young soldiers that the lymphatic tissue of the spleen was best developed in his youngest group (19 and 20 years), and least well developed in those over 41 years of age. The excellent quality of this material, however, is considerably offset by his inability on field service to do more than estimate the size of the follicles macroscopically.

By far the most valuable work in this field is Hellman's⁶ (1925-1926, with historical review) analysis of 100 cases, divided into 11 age groups, of persons dying sudden violent deaths and proved at autopsy to have no noteworthy lesions of the spleen. This is truly admirable material, all of the individuals dying within 12 hours of the injury (most of them instantaneously, and all but one within 3 hours), and all studied grossly and microscopically and by the same person. They present a striking curve of quick increase of percentage of lymphocytic tissue in the malpighian follicles from birth to an average of about 11 per cent in the 1st

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year of life, rising to a maximum of 18 per cent in the 18th year, with a sharp drop to 13.6 per cent at 25, remaining level till 45, then another drop to below 5 per cent in the oldest case studied (84 years). One hundred cases, however, is a very small number when distributed over 11 groups, especially as the individual "scatter" was great. There were, for instance, 12 cases below 1 year and 20 below 6, leaving an average of 10 cases for each of the other age groups and only 5 cases over 50 years of age. It should also be noted that the figures given represent percentages only and must be taken in connection with the total spleen weights in order to arrive at the actual amount of lymphoid tissue in the spleen at a given age. Furthermore, they represent only the size of the malpighian follicles, the outer edges of which are often open to considerable interpretation, and they include the intrafollicular vessels and all "pale centers," which many now believe not to be made up of cells of the lymphocytic series. Although the short interval between injury and death precludes changes secondary to wasting, sepsis, and so on, the considerable splenic pulp change that results quickly after shock or hemorrhage cannot be excluded and remains a variable affecting the percentage that cannot be accounted for. We therefore thought that a similar study would be desirable, even though our series is also open to some of the above objections, hoping that larger numbers, especially in the older groups, would throw further light on the subject.

METHODS

We have used human material * from 300 cases of violent death, excluding individuals who were found either from the history or the postmortem examination to have any recognizable disease. As Hellman ⁶ has shown that malpighian follicles are distributed with great uniformity throughout the spleen, and as we were able to confirm this in 1 extensively studied case, we have in each case been content with 1 or 2 sections from the convex surface of the organ and prepared hematoxylin-eosin stained sections of 6 μ thickness. The percentage of lymphatic tissue was obtained by projecting low power ($\times 8.6$) microscopic fields on 8½ by 11 inch paper, under fixed conditions. The outlines of the malpighian follicles

* Obtained with the kind assistance of Drs. Helpern of New York, Werne of Jamaica, L. I., and Wadsworth and Crane of Philadelphia.

were traced and also their contained non-lymphatic tissue (central artery and pale staining reaction centers noted by Hellman) and both areas measured with a planimeter. The percentage of the area of the follicle as a whole is termed the "gross" percentage (*i.e.*, white pulp), and that of the follicle minus the central artery and pale center, the "net" percentage. When a marginal zone (*aussenrandzone*) of the follicle, where the lymphocytes are less concentrated, was found, it was not included in the area measured, as better measurement was possible where the much sharper transition occurred at its inner edge than where it gradually merged into the red pulp. In fact, not infrequently the transition to the red pulp was so gradual that a line of demarcation would have been a purely arbitrary one. This of course means that our percentages do not represent the actual amount of lymphatic tissue in the follicles, but this is also true of other studies that have included artery and pale centers in the follicular area. Furthermore, the true lymphocytic content of the spleen as a whole cannot be measured in any case because of the considerable number of lymphocytes both in the *Lymphscheiden* (lymph sheaths) and scattered through the red pulp. Our method also provides for obtaining a figure for the number of malpighian follicles per unit area. Furthermore, the weight of the spleen being known, an approximate value, limited by the considerations given above, can be obtained for the weight of the lymphatic tissue in the spleen; and, the body weight being known, the ratio of this lymphatic tissue weight to body weight may be determined (Chart 2). At first 10 microscopic fields per spleen section — each field representing approximately 5 sq. mm. of splenic tissue — were deemed sufficient for examination. A study of 200 fields from 10 different parts of 1 spleen, however, demonstrated that the variation from field to field might be considerable and that measurements of 20 fields from each case were required to furnish reliable averages. Like Hellman, we found no irregularities of malpighian follicle distribution in the sections of the spleen more extensively studied. It was hoped at first that this study could be based on 500 cases, but the extra material and labor that was required for the more intensive study of each case, together with a necessarily restricted time element, has limited us to 300 spleens. In most of the age groups, however, a satisfactory number of cases has been afforded, though

at the two extremes of life larger numbers would have been useful. In some of the earlier sections studied the small amount of tissue available did not permit a 20-field study — an unavoidable defect in the material. We of course recognized that our results might be unduly influenced by the cases that were studied over the smaller areas. To control this possibility the mean percentages of the

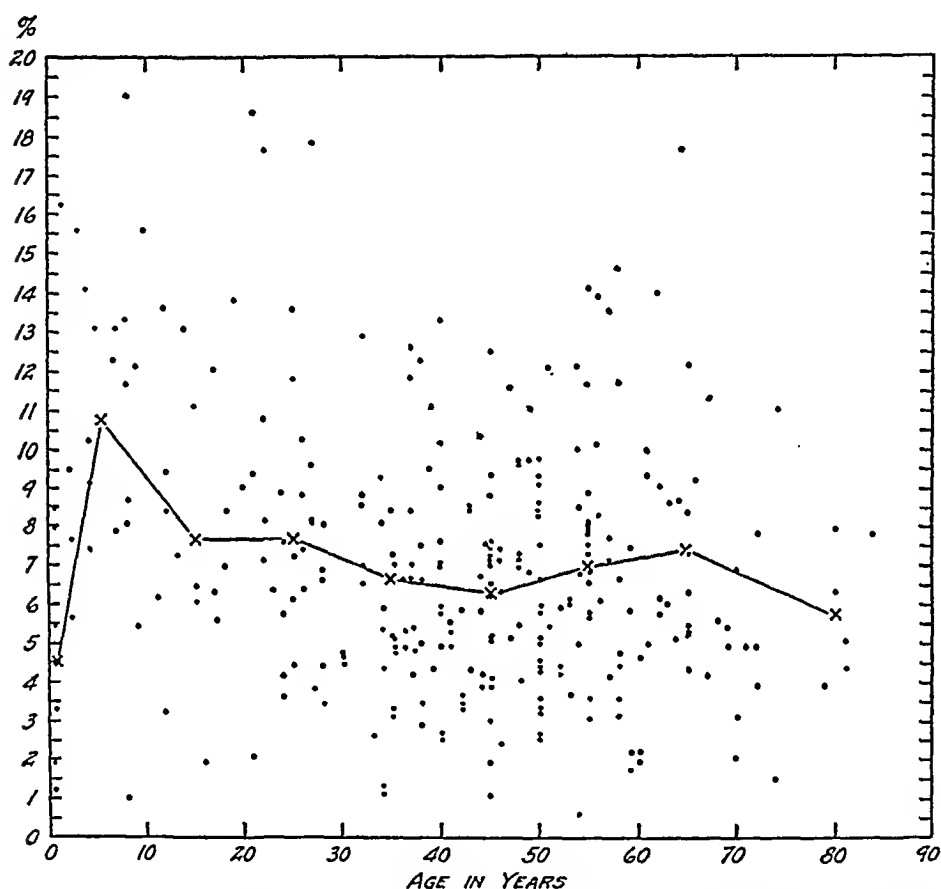


Chart 1. "Net" percentages of lymphatic tissue in the malpighian follicles at different ages in 300 cases of violent death. Dots indicate individual cases and curve of the mean for each decade.

10-field cases were combined with the 20-field cases "weighted" so that the latter had twice the value of the former. The resultant curve was so closely parallel to the unweighted curve, however, that the latter was adopted without further ado.

RESULTS

The individual "net" percentages of lymphatic tissue, obtained as described above in the 300 cases, have been charted individually and a mean curve drawn for the 9 age groups (Chart 1).

This curve shows that, as in Hellman's series, the percentage of lymphatic tissue as studied by us is small in infants (4.5 per cent), but quickly rises to a maximum in the 1st decade (10.8 per cent). This was reached about 10 or more years earlier than the age of maximum weight of the spleen, as found both in this and in our earlier study.¹ The curve then drops sharply to 7.7 per cent in the 11 to 30 age groups, tending to fall gradually in the next 2 age groups. After a slight rise in the 2 age groups from 50 to 70

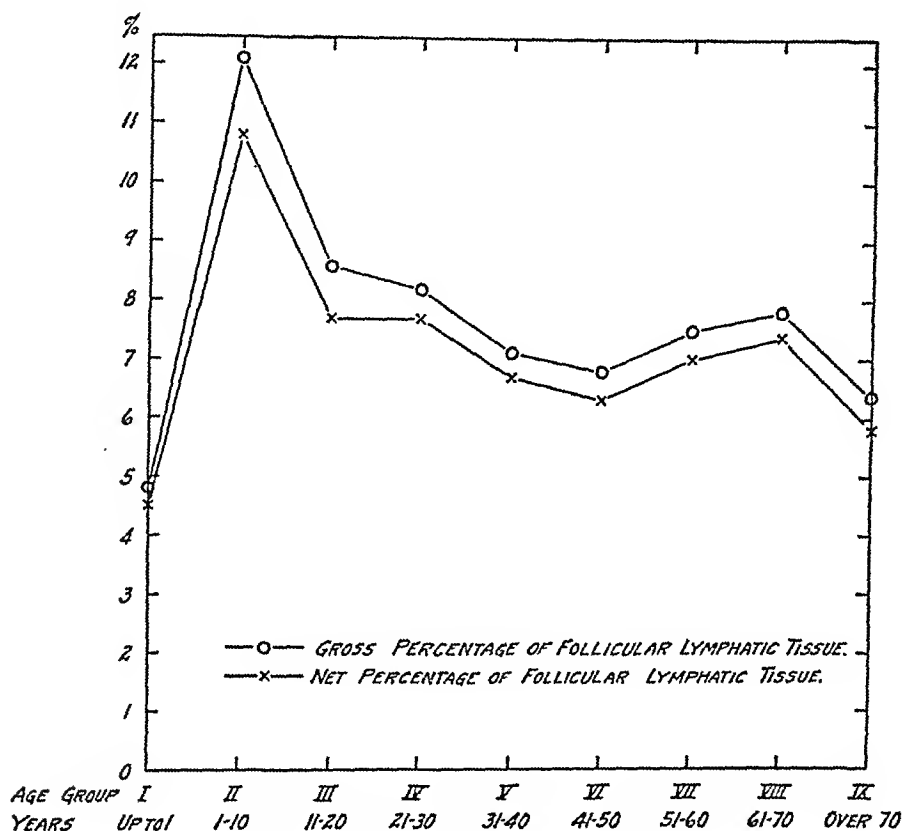


Chart 2. "Net" and "gross" percentages.

years, it falls in the last group (comprising all cases over 70) to 5.8 per cent. When studied statistically in terms of the standard error and regression coefficient, it is found: (1) that the peak at the 1 to 10 year period is significantly higher than the level of the period on either side. (2) If the groups from 11 years on are considered, the regression coefficient shows that the line is not significantly different from horizontal, though it does have a slightly downward trend. (3) If the regression line is considered in three parts (*i.e.*, from 11 to 50 years, from 41 to 70 years, and from 70

TABLE I

Data on the Lymphatic Tissue of Malpighian Follicles at Different Ages in 300 Cases of Violent Death

Group	Age	Number of cases	Net percentage \pm 2SE	Gross per cent	Weight of follicular lymphatic tissue	Ratio $\frac{\text{Follicular lymphatic tissue weight}}{\text{Body weight}}$	Number of malpighian follicles \pm 2SE
1	yrs. Up to 1	7	4.5 \pm 2.0	4.8	gms. 0.36	0.00019	5.6 \pm 0.62
2	1-10	22	10.8 \pm 1.8	12.1	6.7	0.00030	4.2 \pm 0.44
3	11-20	18	7.7 \pm 1.6	8.6	10.6	0.00019	3.5 \pm 0.44
4	21-30	36	7.7 \pm 1.2	8.4	10.6	0.00016	3.2 \pm 0.24
5	31-40	54	6.7 \pm 0.8	7.1	8.5	0.00014	3.3 \pm 0.34
6	41-50	68	6.3 \pm 0.6	6.8	9.0	0.00012	3.0 \pm 0.22
7	51-60	53	7.0 \pm 1.0	7.5	9.2	0.00013	3.3 \pm 0.42
8	61-70	29	7.3 \pm 1.0	7.8	8.9	0.00014	3.9 \pm 0.54
9	Over 70	13	5.8 \pm 1.2	6.3	5.7	0.00010	2.8 \pm 0.54

years on), three limbs result. The first of these is significantly downward, the second significantly upward and the third significantly downward. With proper regard for the above items 2 and 3, it can only be said that there is a definite suggestion of an increase in lymphatic percentage at ages 50 to 70, followed by a definite decline. This increase is not sufficiently marked, however, to rule out the possibility that it is merely an irregularity in a trend of a curve which from 11 years on is gently but steadily downward.

By subtraction of the "net" from the "gross" percentage of lymphatic tissue in the 9 age groups (Chart 2), figures can be obtained for the not inconsiderable areas occupied by the "central arteries" and the "pale centers." Although the effort was not made to separate these two factors and although there is still controversy as to whether "pale centers" represent lymphopoietic or reticuloendothelial tissue, or both, yet the higher percentages for the combined areas in early life are suggestive of greater lymphopoietic activity at that period — a finding already reported by earlier writers. It is hoped that this matter can be studied in proper detail in the near future and perhaps more light thrown on this phase of the ageing of splenic lymphatic tissue.

When the absolute weight of the lymphatic tissue in the malpighian follicles is estimated by multiplying the individual percentages of lymphatic tissue by the spleen weights, a somewhat different situation is encountered (Chart 3). The maximum sharp peak shifts from the 2nd group (1 to 10 years) to the 3rd and 4th groups (11 to 30 years), and the low secondary peak, which was present in the 7th and 8th groups (51 to 70 years) in the per cent chart, is modified by an increase also in the 6th group (41 to 50 years), so that this part of the curve suggests a maintenance or even a slight increase of splenic lymphatic tissue from 30 to 70 years.

Estimation of the ratio of the means of the absolute weight of the lymphatic tissue of the malpighian follicles to the means of the body weights (Chart 3) results in a curve much like that of the percentage of lymphatic tissue (Chart 1), except that the peak in the 1st decade is more marked. This, like the absolute weight estimates, then, tends to support the suggestion that there may be a later increase of lymphatic tissue — absolute as well as relative

— in the spleen lasting till old age, though it is still far from being statistically proved.

The number of malpighian follicles per unit area was found to be greatest in the 1st year (actually the first 3 months after birth) *i.e.*, a mean of 5.6 follicles per unit microscopic field (see Chart 3). These follicles, however, were very small, as must be the case in view of the low lymphoid percentage found at this period. The number of follicles per unit area decreased in each group to the age

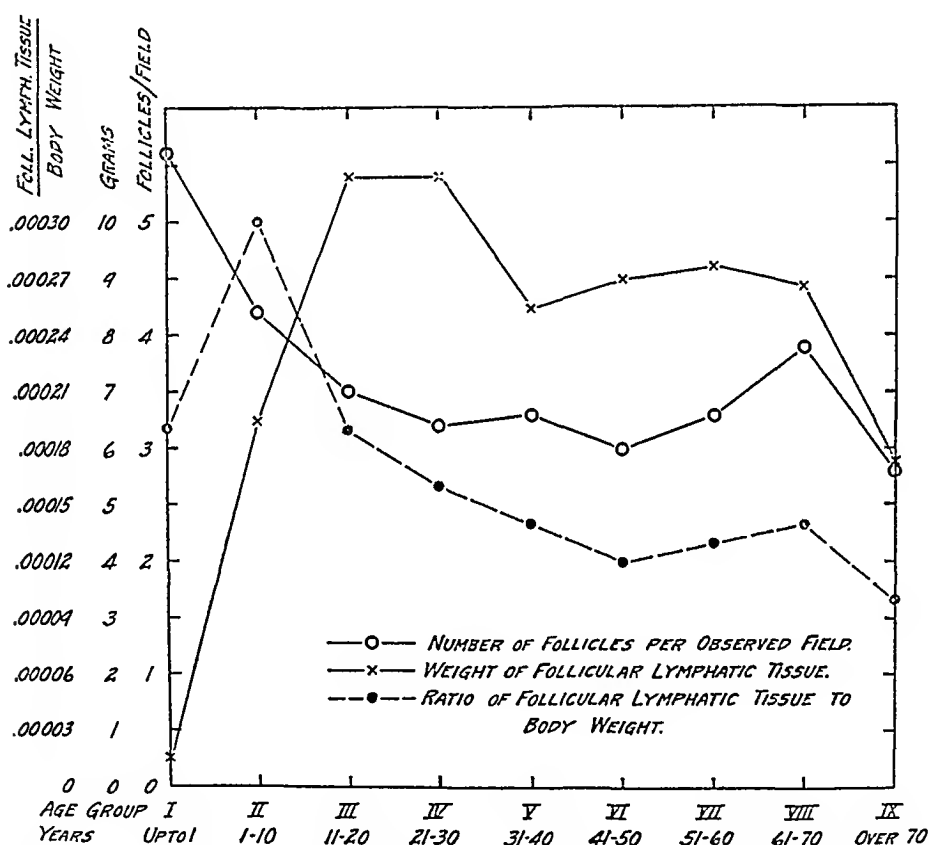


Chart 3. Weight of lymphatic tissue in the malpighian follicles, its ratio to body weight and the number of malpighian follicles.

of 30 (3.2 per field), then maintained a fairly constant level without significant changes, except for a rise in the 8th decade (3.9 per field) and a terminal drop (2.8 per field). These statements, of course, deal only with the concentration of follicles. It has not been possible to investigate their total number in the spleen at different ages; but in view of the much greater increase in the size of the adult organ than decrease in the number of follicles per unit

area, it is probable that their total number is also increased in the adult.

Comparison of the age, sex, color, mean spleen weight and mean body weight of these 300 cases with similar records of 2000 violent deaths (Krumbhaar and Lippincott¹) brings out no significant differences. There is reason for regarding both series as not far from an average sample of the population in the eastern United States. In the smaller series, as is to be expected, there are more variations, but they are so unimportant that they will not be considered further.

DISCUSSION

It will be seen that in our series each of the 6 adult groups up to 70 years of age contains 29 cases or more, and that there are 95 cases analyzed over 50 years of age, as compared with the 7 cases over 50 in Hellman's series.

In Hellman's series the percentage of follicular lymphatic tissue exhibited a steadier drop from the maximum than did ours, and showed higher values throughout (his Table 18). At least two factors in making our percentages smaller than those of Hellman are: first, the different points taken as the margin of the follicle; and second, the exclusion of central artery and pale reaction centers from the "net" areas. Consideration of our gross curve indicates that the second factor is of lesser importance. Hellman's curve for the absolute weight of the follicular lymphatic tissue (white pulp) is similar to ours in attaining a maximum later than the percentage maximum but differs in showing no tendency to the secondary increase in later life and again in having constantly higher values. We have not been able to determine that such unavoidable complications as shock or hemorrhage, or a rare mild infection or longer survival of a few days after the violence made any noticeable difference in the amount of lymphatic tissue of the cases we have studied.

The individual variation of the follicular lymphatic tissue at given ages is marked (Chart 1), as might be expected from the study of "normal" spleen weights previously reported,¹ and as found also in Hellman's lymphatic percentages. Unexpectedly low percentages are occasionally found in the younger age periods for which the most careful scrutiny, excluding such factors as

malnutrition, infectious disease, and so on, provides no explanation. Similarly, unexpectedly high percentages may be found in older life, though here the possibility of reaction to unobserved mild infection in some part of the body cannot be overlooked. Such marked individual variation, which lessens the value of statistical treatment of these figures, must apparently be ascribed to the unhelpful "individual idiosyncrasy." A wide normal range must be accepted; Hellman came to the same conclusion.

SUMMARY AND CONCLUSIONS

The amount of lymphatic tissue in the malpighian follicles of the spleens of 300 persons dying violent deaths has been studied at different age periods as part of a general study of the ageing of lymphatic tissue.

The percentage of follicular lymphatic tissue is small in infants but rises to a maximum in the 1st decade of life (*i.e.*, 10 or more years earlier than the whole spleen reaches its maximum weight). After a sharp drop in the next decade, a gentle fall in percentage occurs through the rest of life, with a suggestion of a second increase from age 51 to 70, followed by a distinct drop. No notable difference is found when this percentage is figured on the "net" (*i.e.*, excluding "central" arteries and pale centers) or on the "gross" basis.

The weight of the follicular lymphatic tissue (net per cent multiplied by the postmortem weight of the spleen) reaches a maximum later in life (11 to 30 years) than the per cent maximum, with a lower level maintenance (or even slight increase) until a final fall in the oldest age group.

The curve of the ratio of the follicular lymphatic tissue to the body weight is not strikingly different from that of the percentages.

Malpighian follicles are most numerous per unit area in early infancy, though small. They decrease in number to about the age of 30 years, then maintain a fairly constant number (except for an apparent rise in the 8th decade).

The combined areas of "central" arteries and pale centers are smallest in early infancy and greatest from 1 to 10 years. After decreasing through 3 decades, this area remains unchanged through the rest of life, perhaps because increasing thickness of the blood vessel walls compensates for a decreasing amount of pale centers.

Thus it can be said that the lymphatic tissue of the malpighian follicles in general behaves more or less like the lymphatic tissue elsewhere in the body at different ages. It differs from most of the lymphatic structures, however, in the sharp percentage decrease in the 2nd decade of life to a level that is fairly well maintained through the 7th decade, with even the suggestion of an increase in the 6th and 7th decades, while its absolute weight maintains its maximum through the 3rd decade.

NOTE: We are grateful to Messrs. M. S. Abel, J. D. Bibb, and W. H. Kety of the second year class in this medical school for their aid in outlining and measuring malpighian follicles, and especially to Mr. Abel for his advice and assistance in the statistical phases of the study.

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MULTIPLE NECROSES OF THE SPLEEN (FLECKMILZ)*

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"Multiple necroses" of the spleen is a descriptive term ascribed in the literature to a peculiar, characteristic gross appearance of the organ in which there occur multiple, large and small, bizarre shaped, map-like or rounded areas of firm, yellowish to grayish white necroses. These occur as isolated changes or are connected with one another and have infarct-like borders. In contrast with the ordinary embolic infarction of the spleen multiple necroses have been observed only infrequently. A survey of the literature would suggest that they are of varied origin.

An impetus was given to the study of this occurrence by Feitis¹ in 1921 who reported 2 cases and called the condition "Fleckmilz," or speckled spleen. The widespread interest created by this article has resulted in the report in the literature of 27 cases up to the present time. It is of interest that all of the spleens were encountered at autopsy and there was no suspicion clinically of the true process present since no pathognomonic signs or physical symptoms were noted.

Because of the current interest in the entity and the comparative rarity of reports in the literature of multiple necroses of the spleen, the following 2 cases are placed on record. Each of these differs etiologically from the cases reported by Feitis.¹ There is included, also, a comparison of the cases reported from a clinical, etiological, and pathological standpoint, in so far as generalization of a few cases permits.

CASE REPORTS

CASE 1. J.J.B., a white male, aged 17 years, was admitted to the medical service of the John Gaston Hospital Nov. 28, 1936, complaining of general malaise, severe headaches and chilly sensations of 8 days duration. Two days following the onset of illness he experienced a severe chill and began passing dark brownish urine, which continued for 3 days. The symptoms of headache and malaise continued with high fever, nausea and weakness.

Physical examination revealed a well developed and nourished white male

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who was somewhat disoriented as to time and place. The temperature was 102° F., pulse 140 and respirations 20 per minute. The blood pressure reading was 122/40. There was an icterus of the skin and a generalized lymphadenopathy. No other abnormality was noted.

Laboratory examination revealed a red blood cell count of 1,260,000, white blood cells 6950, and hemoglobin 4 gm. per cent. Differential study showed a lymphocytosis. The urine was alkaline and cloudy with a specific gravity of 1.010, 3 plus albumin, and 3 to 6 granular and hyaline casts per high power field. The Kahn blood test was negative. Blood smears examined for malaria were negative. Blood cultures were positive for *Bacillus paratyphosus B* on repeated examination.

The clinical course continued to indicate a septic condition with the temperature varying between 99 and 105° F. Delirium became more marked, supportive treatment was of no avail, and the patient died Dec. 3, 1936, on the 6th day of hospitalization.

Postmortem Examination

Autopsy was performed 2 hours following death and revealed a well developed and nourished, anemic, jaundiced young white male. Examination of the brain showed numerous, tiny, 1 up to 3 mm. sized petechiae within the substance of the cerebral cortex. On microscopic examination these were found to be small capillaries occluded by thrombi and surrounded by hemorrhage. The lungs were firmer than normal and oozed copious amounts of straw colored fluid from congested sectioned surfaces. The remaining viscera revealed parenchymatous degeneration.

In gross the spleen offered a startling picture, being enlarged to 740 gm. The splenic vessels at the hilus had smooth linings and contained fluid blood. The organ was soft in consistence, smooth for the most part, and was violaceous in color. Beneath the capsule there were visible multiple, slightly raised, irregular, rounded or map-like, pin-point up to 3.5 cm. sized, gray to grayish yellow areas surrounded by a broad zone of hemorrhage (Fig. 1). On section the picture was enhanced on a dark red background. The infarcted areas were diffusely distributed through the splenic pulp, presenting a mosaic pattern of isolated and confluent necroses. The larger areas were often connected by narrow bands of a similar necrotic appearing tissue. The smaller areas were isolated. The changes were most marked toward the periphery of the spleen, although they were present irregularly throughout, seeming to affect selectively no particular portion. Cultures from several regions of the tissue revealed *Bacillus paratyphosus B*.

Microscopic Examination

In the spleen it was seen that the grayish white areas represented necrotic splenic pulp in which the outlines of tissue elements could still be recognized. These anemic infarcts were fresh and were large or small in size, often connected by bridges of necrotic tissue with normal intervening splenic tissue. In the larger areas of necrosis the centers showed a pale, eosin staining, homogeneous material. A fine nuclear dust was found to be abundant, particularly with the Heidenhain-van Gieson stain. With this stain a fairly well preserved trabecular and reticular network was demonstrated. Numerous small clumps of bacteria were present (Fig. 2). Occasionally seminecrotic blood vessels were seen, frequently filled with fresh thrombi. At the periphery the tissue was found to be better preserved and was infiltrated with lymphocytes, large mononuclear phagocytes and a few polymorphonuclear leukocytes. This zone merged into a surrounding broad area of hemorrhage. In the smaller zones and in areas in which the degeneration was not so advanced, as well as in the connecting bridges between the larger foci, the above described distinct zones could not be made out. In these latter areas many intact cells were visible, the pulp was seminecrotic, and cells with pyknotic and karyorrhetic nuclei were also seen. In the non-necrotic tissue the pulp was normal. The sinusoids were empty or distended with well preserved red blood cells, lymphocytes and phagocytic mononuclear cells containing iron pigment and a few leukocytes. The germinal centers in the non-necrotic regions showed moderate hyperplasia. The distribution of the necroses suggested that there was no selective involvement of the malpighian bodies. Often a middle sized or smaller artery was seen to contain a thrombus. The trabecular and follicular arteries infrequently showed thrombotic occlusion. Interstitial fibrosis or an increase in reticulum was not observed.

Anatomical Diagnoses: *Bacillus paratyphosus B* septicemia with embolic phenomena in the brain and spleen (Fleckmilz), pulmonary edema, and parenchymatous degeneration of viscera.

CASE 2. Mrs. H.C., a white female, aged 20 years, was admitted to the Evanston Hospital, Chicago, Ill. on March 22, 1930, complaining of having had an abscessed right upper molar tooth for 1 year. This lesion was accompanied by swelling and tenderness of the gums, and later there had been evidence of Vincent's angina. She had slowly but progressively lost weight,

was easily fatigued, and often suffered nausea and abdominal distress following meals. For 5 days prior to admission she had experienced a nocturnal rise in temperature, which subsided each morning. The family and past histories were irrelevant.

Physical examination showed that the temperature on admission was 100° F., and thereafter septic in type, varying between 99 and 106°. The pulse fluctuated between 84 and 150 per minute. Respirations were 18 up to 30 per minute. The patient was undernourished, anemic and jaundiced, and showed evidence of recent loss of weight. The gums over the right upper molar teeth were swollen, tender and ulcerated. The pharynx showed catarrhal hyperemia. The heart and lungs were normal. There was tenderness in the upper abdomen over the region of the spleen and liver.

Laboratory examination revealed red blood cells to be 2,300,000, white blood cells 8000 and hemoglobin 40 per cent. The differential count showed 46 per cent polymorphonuclear neutrophilic leukocytes and 54 per cent lymphocytes. Blood cultures were negative. Cultures from the abscessed molar teeth revealed *Streptococcus viridans*. Smears from the same region showed abundant *Borrelia vincenti*, together with a fusiform bacillus.

During the clinical course repeated transfusions and supportive measures were of no avail. The septic condition continued progressively worse until death on April 8, 1930, on the 16th day of hospitalization.

Postmortem Examination

The body was well developed but undernourished. Pallor and icterus of the mucous membranes were noted. Multiple petechial hemorrhages occurred in the skin over the chest and abdomen. An ulcer was found in the swollen gums along the buccal margins of the right upper molar teeth. The serous cavities contained a slight excess of clear, straw colored fluid. The lungs showed evidences of edema and terminal bronchopneumonia. The remaining viscera revealed parenchymatous degeneration, with the exception of the spleen.

In gross the spleen was markedly enlarged and weighed 918 gm. Through the capsule, and particularly on section, one could see the red pulp studded with multiple, small and large, up to 1 cm. in diameter, irregularly rounded, firm, dark red opaque areas, and one large, map-like grayish yellow area surrounded by a zone of bright red and measuring 4.5 by 1.5 cm. (Fig. 3).

Microscopic Examination

The larger grayish yellow infarct was similar in appearance to those described in Case 1, with a few additional features. The area of necrosis showed a rather marked tendency toward fibrous tissue organization, being particularly prominent in the surround-

ing hemorrhagic zone, as shown by the van Gieson stain. More centrally there was newly formed fibrous tissue. The trabecular and reticular network showed degeneration in the central portion of this larger anemic infarct. Many larger, as well as middle and smaller sized blood vessels showed thrombi with a tendency toward organization (Fig. 4). The smaller areas of tissue necrosis, however, presented the picture of hemorrhagic infarct and appeared to be of more recent origin. In the necrotic portions there was massive hemorrhage both within and without the confining sinusoids so that the splenic tissue for the most part was obscured. The malpighian bodies were made out with difficulty. Culture of splenic tissue showed *Streptococcus viridans*.

Anatomical Diagnoses: Abscessed molar teeth with *Streptococcus viridans* septicemia, multiple necroses of spleen (Fleckmilz), anemia, jaundice, pulmonary edema and bronchopneumonia.

Comment

The above described cases showed a bacterial thrombotic occlusion of the splenic vascular system with resultant multiple infarcts of the organ. These necroses were of a size and distribution varying with the anatomical method of division of the blood vessels as well as the size of the vessel haphazardly the site of a thrombus. The gross appearance of the spleens was similar to the multiple necroses described in the cases of Feitis, but had a different etiological factor. The other cases reported in the literature showed a varied causation but had a similar microscopic appearance.

DISCUSSION

Various writers on the subject of Fleckmilz have at different times attempted to divide or classify the cases reported on the basis of the essential lesion which brings about the necroses. The origin of the necrosis *per se* may be explained in one of three ways, namely closure of the vessel to a designated region of tissue, relative vascular insufficiency with superimposed tissue injury, or pure tissue injury which by accident was situated in the region supplied by the individual vessel. The cases which have been described may arbitrarily be divided into the groups listed in Table I.

All cases reported listed in Table II show the group into which

they may be placed, as well as a comparison of the basic anatomical changes that brought about the infarcts.

Nineteen cases are observed to be similar to those originally described by Feitis¹ and are associated with renal insufficiency. He ascribed the necrosis to arterial damage superimposed upon an arteriosclerosis. The lesions affected primarily the smaller middle sized arteries and consisted of hyaline and fatty degeneration with intimal proliferation and often subsequent thrombosis. Lubarsch⁷ directed attention to the additional factor of the toxin liberated in uremia and renal insufficiency with which these cases were associated. He believed, therefore, that in his 3 cases the thrombosis was due to an autointoxication. The toxin acted upon the arterio-

TABLE I

Multiple Necroses of the Spleen (Fleckmilz). Groups into which Reported Cases may be Divided

Group	Number of cases
1. Arteriosclerotic toxic-thrombotic	19
2. Angiospastic toxic-thrombotic	2
3. Purely toxic	3
4. Arteritic	1
5. Infectious toxic-thrombotic	4
Total	29

sclerotic, relatively inefficient vessels to produce thrombosis. Meuret,⁵ Hosoi,⁸ Nicod,⁹ Klemperer and Otani,¹⁰ Adolphs,¹¹ Spier,¹² Rake,¹³ Laufer,¹⁴ and Guttman¹⁵ agree with this view and cite cases in point.

Geipel² and Matthais³ individually studied spleens with extensive isolated and aggregated infarctions in which there was considerable arterial and venous thrombosis, but in which the vessel walls were normal. The organs were from patients who died from eclampsia. They agreed with Beneke's idea (quoted by Matthais) of the origin of organic injuries through angiospasm caused by the hypothetical eclampsia toxin and followed by secondary thrombosis. These, then, are classed as the angiospastic toxic-thrombotic group.

Enzer,⁶ Lubarsch,⁷ and Magnus¹⁶ each described spleens from individuals who suffered from profound anemia but no changes were noted in the blood vessel walls, either thrombosis or degener-

TABLE II
Multiple Necroses of the Spleen (Flecknitz). Cases Reported to Date

Author	Age	Sex	Spleen	Basic pathology	Group
Feitis (1921)	yr.s. 39	M	205 gm.	Chronic nephritis, uremia, arteriosclerosis	1
Feitis (1921)	60	M	8 x 4.5 x 1.5 cm.	Chronic nephritis, arteriosclerosis	2
Geipel (1924)	35	F	330 gm.	Eclampsia, renal cortical necrosis	2
Matthais (1924)	..	F	..	Eclampsia, renal necrosis, brain hemorrhage	1
Meuret (1924)	31	M	12 x 7 x 4.5 cm.	Chronic nephritis, uremia	1
Meuret (1924)	46	M	..	Arteriosclerosis, renal insufficiency, brain hemorrhage	5
Wilton (1925)	31	F	650 gm.	Upper respiratory infection, empyema sphenoid	3
Enzer (1926)	59	F	9 x 5 x 3 cm.	Pernicious anemia	1
Lubarsch (1927)	44	F	105 gm.	Generalized arteriosclerosis, renal insufficiency	1
Lubarsch (1927)	52	M	130 gm.	Generalized arteriosclerosis, renal insufficiency	3
Lubarsch (1927)	215 gm.	Generalized arteriosclerosis, renal insufficiency	1
Lubarsch (1927)	51	M	..	Pernicious anemia	1
Hosoi (1928)	45	F	110 gm.	Chronic nephritis, arterio-sclerosis, apoplexy	

TABLE II (Continued)

Author	Age	Sex	Spleen	Basic pathology	Group
Nicod (1930)	yrs. 38	M	14 x 9 x 4 cm.	Chronic nephritis, uremia, arteriosclerosis	I
Nicod (1930)	48	F	250 gm.	Chronic nephritis, uremia	I
Klemperer and Otani (1931)	46	F	..	Chronic nephritis, uremia	I
Adolphs (1931)	33	F	..	Chronic nephritis, arteriosclerosis	I
Adolphs (1931)	57	M	..	Chronic nephritis, arteriosclerosis	I
Adolphs (1931)	11	F	..	Nephritis, uremia	I
Spier (1931)	51	F	92 gm.	Arteriosclerosis, renal failure, brain hemorrhage	I
Rake (1932)	44	F	140 gm.	Chronic nephritis, uremia	I
Laufer (1933)	58	M	13 x 7.5 x 5 cm.	Chronic nephritis, uremia	I
Laufer (1933)	37	M	15 x 8.5 x 4.5 cm.	Chronic nephritis, uremia	I
Laufer (1933)	26	M	15 x 15 x 12 cm.	Streptococcic septicemia, appendiceal abscess	5
Guttman (1934)	45	F	40 gm.	Arteriosclerosis, renal insufficiency, pneumonia	I
Magnus (1937)	21	M	600 gm.	Necrotizing arteritis	4
Magnus (1937)	63	M	300 gm.	Anemia, pneumonia	3
Schmeisser and Harris (1938)	17	M	740 gm.	<i>B. Paratyphlosis B.</i> septicemia	5
Schmeisser and Harris (1938)	20	F	918 gm.	<i>Streptococcus viridans</i> septicemia	5

ation. These are the types that are probably due to pure tissue injuries which were by chance located in areas supplied by individual arteries, and the etiological factor is purely toxic.

Magnus' 2nd case yielded a surprise. No similar record can be found. He ascribed the tissue necrosis to a widespread acute necrotizing arteritis resembling somewhat periarteritis nodosa but in which there was an absence of periarteritic leukocytic infiltration and aneurysm formation. The necrosis was manifest, not only in the spleen but in the pancreas, kidneys and alimentary tract. This, then, exemplifies a type different from any other. It may be grouped as arteritic in nature.

Our 2 cases comprise still another group from the standpoint of etiology. They must be classed as due to infection with the possibility of the toxins liberated coming into play. Wilton's ⁴ description of a patient with empyema of the sphenoid sinuses, associated with an acute upper respiratory infection, probably belongs to this group. Laufer's ¹⁴ 3rd case was from a man who had a streptococcic septicemia incident upon an appendiceal abscess, and closely resembled our 2nd case.

SUMMARY

1. Fleckmilz (Feitis¹) is a descriptive term applied to the gross appearance of a spleen with multiple infarcts and necroses.
2. The cases subsequently reported in the literature have had a similar macroscopic appearance but have been of varied origin.
3. It is observed that the multiple necroses can be divided, etiologically and pathologically, into five groups.
4. We report 2 cases, which are classed in the infectious toxic-thrombotic group, the 1st showing anemic and the 2nd mainly hemorrhagic infarcts.

NOTE: We are indebted to Dr. W. W. Brandes for permission to use the 2nd case and to Dr. Joseph L. Scianni for the illustrations.

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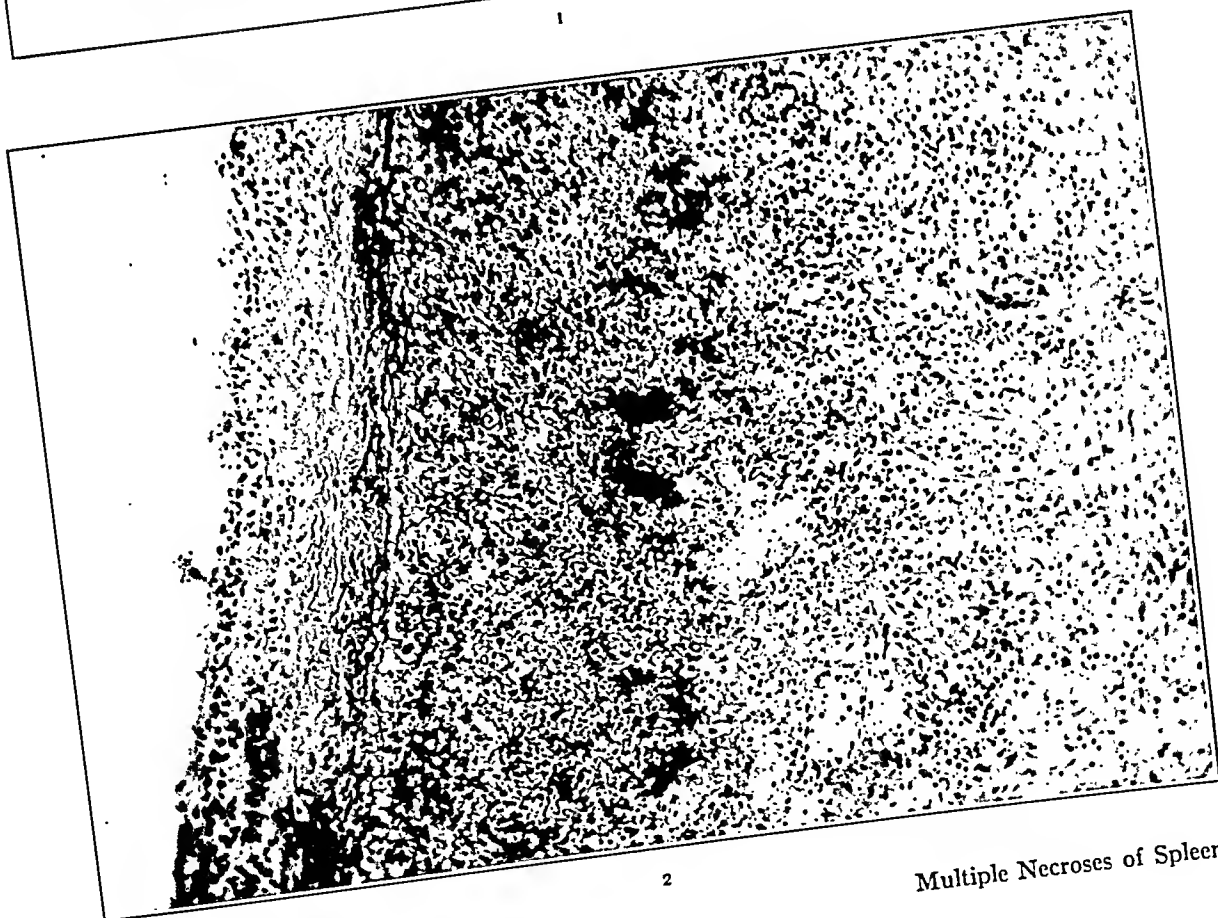
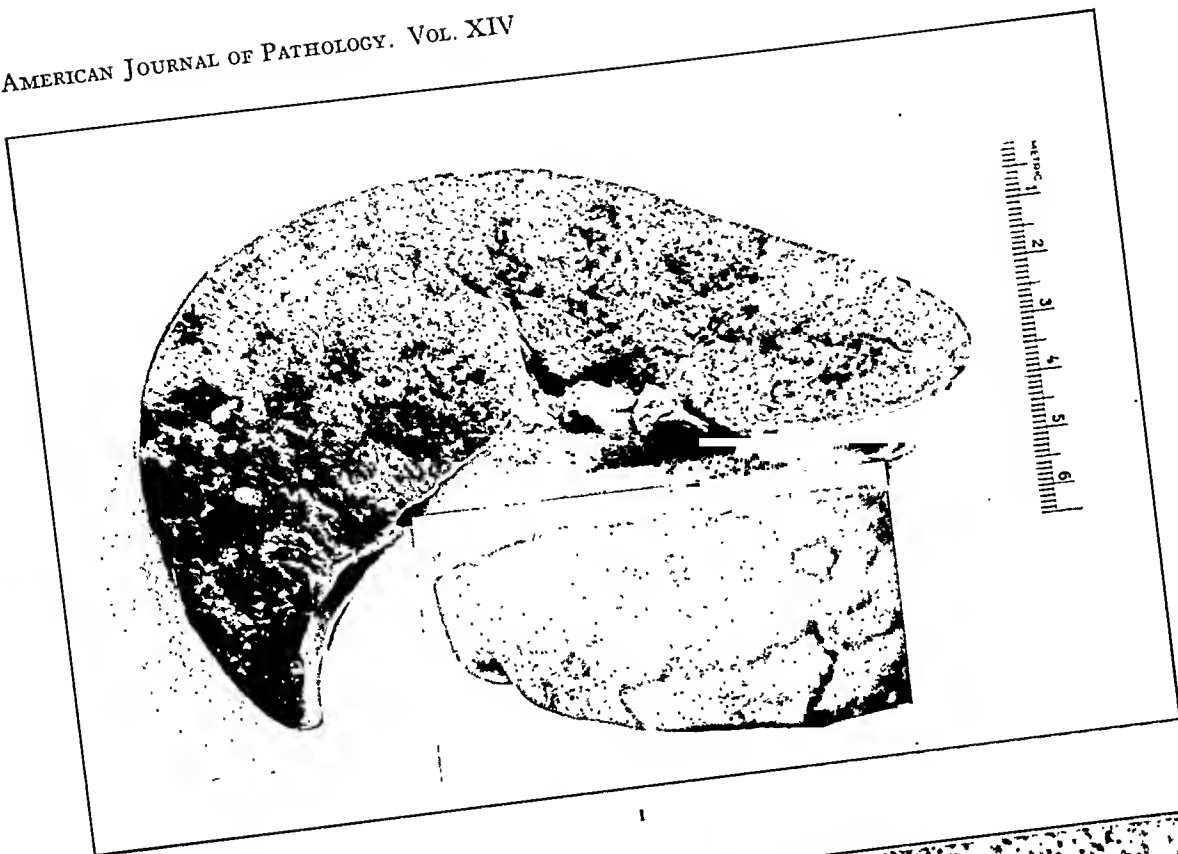
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DESCRIPTION OF PLATES

PLATE 152

FIG. 1. Case 1. Multiple necroses of the spleen (Fleckmilz). The enlarged spleen shows beneath its capsule and on section multiple, slightly raised, map-like, gray to grayish yellow areas surrounded by a broad zone of hemorrhage.

FIG. 2. Case 1. Multiple necroses of the spleen (Fleckmilz). Part of an anemic infarct is seen to the right, margined by small clumps of bacteria and better preserved tissue infiltrated with many red blood cells and a few lymphocytes, large mononuclear phagocytes and polymorphonuclear leukocytes. At the left is the living capsule. Microphotograph $\times 200$.



Multiple Necroses of Spleen

Schmeisser and Harris

PLATE 153

FIG. 3. Case 2. Multiple necroses of the spleen (Fleckmilz). The markedly enlarged spleen seen on section is studded with irregularly rounded, firm, dark red opaque areas and one large, map-like, grayish yellow area surrounded by a red zone.

FIG. 4. Case 2. Multiple necroses of the spleen (Fleckmilz). Portion of a hemorrhagic infarct with the thrombosed artery is shown surrounded by living splenic tissue at the left. Microphotograph $\times 200$.



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GIANT INTERSTITIAL CELLS AND EXTRAPARENCHYMAL INTERSTITIAL CELLS OF THE HUMAN TESTIS *

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The frequent presence of interstitial cells of the testis (Leydig cells) with 2 or 3 nuclei is often mentioned in the literature, but the finding of multinucleated giant interstitial cells with up to 20 and 30 nuclei has to my knowledge never been described or illustrated, although it has been vaguely hinted.

It is of interest that the original description of the interstitial cells by Leydig ¹ in 1850 contains illustrations of binucleated cells. Von Winiwarter ² in one of the classical papers on the histology of the interstitial cells illustrated an interstitial cell with 4 nuclei but did not discuss the number of nuclei and in fact mentioned it only as incidental to the increased number of centrosomes. Rasmussen ³ states that binucleated and even multinucleated cells have been described and uses von Winiwarter's figure as an illustration. Maximow ⁴ states only that cells with 2 nuclei are relatively common. Cowdry ⁵ says that 2 or even more may occur in a single cell. Oberndorfer ⁶ states that cells with 2 or 3 nuclei are often found and illustrates cells with 4 and 3 nuclei. Wieser ⁷ "not seldom" found cells with 2 and more nuclei, but said no more. In none of the above cited articles has the "and more" in connection with the number of nuclei been further elucidated. Stieve ⁸ comes the closest to mentioning a giant interstitial cell when he says that often one finds 2 and more nuclei lying close together in a mass of cytoplasm (Plasmabezirk), but he does not illustrate this. Nowhere, then, in the literature has anyone definitely spoken of or illustrated an interstitial (Leydig) cell of the testis with more than 4 nuclei.

With no very minute degree of searching I was able to find giant interstitial cells with 4 or more nuclei, up to as many as 30, in 85

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of 721 microscopically sectioned testes.* Cells with 2 and 3 nuclei were of course seen frequently, but these were not counted. The number of giant cells per testis varied from 1 in about 10 low power microscopic fields, which was considered the minimum number necessary to include the testis in this group, to about 10 in 1 low power microscopic field. The range of nuclear multiplicity was complete; that is, cells could be seen with 4, 5, and so on, up to 10, 15, 20 or 30 nuclei; the number most frequently seen was 8 or 10. Nuclear division, mitotic or amitotic, was not seen. In about six instances cells with 3 or 4 nuclei were seen among the extraparenchymal interstitial cells in the tunica albuginea and hilus testis, and one cell with 8 nuclei (Fig. 4) was seen here. Neither giant nor ordinary interstitial cells were seen in the epididymis.

These giant cells are excellently illustrated in the microphotographs (Figs. 1, 2, 3 and 4). They occur both isolated and in the midst of small groups of mononuclear interstitial cells, from which they are unquestionably derived. They are usually oval and measure up to about 50 by 80 . The nuclei, grouped more or less in a semicircle at one or both ends of the cell, are usually identical with those of the mononuclear interstitial cells, but often in the larger cells (Figs. 2 and 3) they are smaller, darker and more wrinkled. One fairly prominent nucleolus is usually seen. The cytoplasm is eosinophilic and somewhat granular. A short distance in from the border of the cell, between and inside of the nuclear semicircles or masses, is a zone of brownish orange pigment similar to that in the mononuclear cells; inside this is an oval or spherical center zone of clearer cytoplasm (Figs. 1, 3 and 4). Reinke crystals were observed rarely in cells with 4 nuclei, but not with higher numbers (they were found, without special search, in the ordinary interstitial cells of about 40 of the 721 testes).

Whether these giant cells form from fusion of several pre-existing single cells or by nuclear division and enlargement of one cell could not be determined. There appears to be no correlation

* The 721 testes which formed the basis of this study were obtained from 470 routine autopsies on persons 18 years of age or older, performed by myself or other members of the Department of Pathology of the University of Minnesota. The primary object of the study was to determine the changes in the weight and histological structure of the adult testis with age and in various diseases. As study of the microscopic sections progressed I was impressed by the frequent occurrence of giant and extraparenchymal interstitial cells.

with age; in the age group 18 to 40 years they were found in 8 testes; 41 to 66 years, in 58 testes; and 67-88 years, in 19 testes. These figures correspond fairly well with the age distribution of the entire series. They were found in all types of disease conditions — 21 cases of acute conditions, 54 of chronic non-malignant, and in 10 malignant. Here again there is no great variation with the distribution of the entire series. As can be seen from the illustrations there is little resemblance between the giant interstitial cells and Langhans giant cells; any possible relationship should, however, be discussed. Of my total material of 470 cases, 16 had tuberculosis as the chief cause of death. Four of these 16 cases showed tuberculous epididymitis; 2 of these 4 also showed tuberculosis in the testis; none of the 4 had giant interstitial cells, although many of the usual (Langhans) giant cells were present. Giant interstitial cells were seen in 3 of the 16 cases with tuberculosis as the chief cause of death, but none of these 3 showed tuberculous involvement of either epididymis or testis.

It was my impression that the giant interstitial cells tended to be present with the more moderate increases in the numbers of the ordinary interstitial cells rather than with massive numbers of the latter or with the normal small quantity. Of the 85 testes showing giant interstitial cells, ordinary interstitial cells of what I chose to call Grade 0 or normal (roughly 50-150 per sq. mm.) were present in 15 per cent, Grade 1 (slightly increased or roughly 150-400 per sq. mm.) in 60 per cent, and Grade 2 (moderately increased, or about 400-1000 per sq. mm.) in 22 per cent. This compares with 40, 43 and 13 per cent respectively for the entire series of 721 testes. The highest degrees of increase, Grades 3 and 4, were present in 3 per cent of the testes with giant cells and in 4 per cent of the entire series. The giant interstitial cells did not tend to be present with severe atrophy; otherwise no definite relation to either diffuse or focal atrophy could be observed.

EXTRAPARENCHYMAL INTERSTITIAL CELLS

Knowledge of this type of cell, occurring in both the testis and the ovary, is quite recent. These cells have hitherto usually been described under the term "sympathicotropic cells." The first accurate description, the name "sympathicotropic," the first mention of their occurrence in the testis, and their first comparison

with the interstitial cells of the testis, was given in 1922 by Berger,⁹ who since then has written the most on this subject. Berger's findings received confirmation in the American literature in 1927 by Brannan,¹⁰ who studied chiefly the ovary, but also found similar cells in 6 testes. Several European authors have confirmed Berger's findings, although some question his interpretations and conclusions.

Berger⁹ described the "sympathicotropic" cells as small or large masses of cells intimately associated with the non-medullated nerves of the ovary and testis. The cells were large, polymorphic and acidophilic, with a finely reticulated nucleus and a large nucleolus; the protoplasm was granular or else compact in the center and clear or foamy at the periphery. The cells were in intimate relation with nerve fiber bundles and sometimes within them; they were also in intimate relation with capillaries. They sometimes contained brown pigment, or crystalloids similar to those in the interstitial cells, or doubly refractile alcohol-soluble lipoids. They were not chromaffin cells but showed what Berger called an attenuated chromaffinity; some of the cells had a natural light brown tint and this became more intense after chromation. Berger later modified this statement concerning chromaffinity. The morphology of the cells in the testis was similar to those in the ovary.

In the testis Berger found the cells around the nerves near and in the testis and in the tunica albuginea, but not in the epididymis. At certain points he found these cellular masses to be continuous with the regular interstitial cells of the testis and having identical morphology. A second important finding was that the variations of the "sympathicotropic" cells paralleled those of the interstitial cells with regard to number, pigmentation, crystals, and so on.

Berger's excellent morphological studies were somewhat overcast by the endocrinological implications he gave to these cells. In his 1922 publication he concluded that these cells were a part of the "interstitial gland" of the testis, following the ideas of Bouin and Ancel¹¹; he gave to the cell masses in the ovary the provisional name of "sympathicotropic gland of the hilus of the ovary," and considered it the homologue of the "interstitial gland" of the testis. Berger's 1928 and 1930 publications^{12, 13} essentially restated his previous findings, and the latter also contained a rather speculative consideration of the "neurocrine" function of the cells

which need not concern us here. In 1932 Berger published ¹⁴ a résumé of the literature, pro and con, to that date; by this time he had also concluded that the "sympathicotropic" cells of the testis were purely and simply Leydig cells, entirely different from chromaffin (paraganglionic, pheochrome) cells, although formerly he had thought that they had some of the features of both. In the interim some opposition to Berger's views had appeared, chiefly from de Winiwarter ¹⁵ who considered these cells to be chromaffin cells.

De Winiwarter (the name is sometimes spelled von Winiwarter) had previously, in 1911, described pheochrome cells in the hilus of the ovary and testis of the fetus and the newborn. After Berger's publication he investigated the adult ovary and reached the same conclusions as previously concerning the fetus and the newborn. Berger's preparations and illustrations, he thought, merely confirmed his own previous work instead of dealing with a different type of cell. He saw no crystalloids and thought that these cells had but a superficial resemblance to Leydig cells; he admitted that he had been unable to obtain the chromaffin reaction. The great difficulty in de Winiwarter's reasoning, however, is that Berger had stressed the resemblance of the "sympathicotropic" cells to the interstitial (Leydig) cells of the adult testis, and de Winiwarter stated that he had not examined the adult testis but was sure the same findings would hold good there as had in the fetus and newborn.

Brannan's paper, ¹⁰ appearing in 1927, is of considerable interest in that it appears to be the only one in the American literature dealing with the "sympathicotropic" cells (all of Berger's publications have been in French and German journals). Brannan studied chiefly the cells in the ovary, but he did find small nodules around non-myelinated nerves in the hili of 6 testes. In the main he agreed with Berger that chromaffin and argentaffin reactions were negative, that the cells contained lipoids, pigments and crystals, that they always occurred in small groups in and near the hilus of the ovary or testis, and that their chief feature was their constant association with nerves. Brannan stated that their association with nerves, together with the known fact that chromaffin (pheochrome) cells did occur in the testicular and ovarian hili, would lead one to suspect that the "sympathicotropic" cells would also possess

chromaffinity, but he could not demonstrate it. He also stated that the cells appeared to be epithelial in nature but not glandular. Brannan made no mention of the regular interstitial cells of the testis in connection with the "sympathicotropic" cells.

Kohn¹⁶ also confirmed Berger's findings and stated that in both the ovary and the testis these cells had all the attributes of Leydig cells — lipoid, pigment, Reinke crystals — and that in the testis their number and characteristics paralleled those of the Leydig cells. Pawlowski,¹⁷ who studied only the ovary, took an attitude intermediate between Berger and de Winiwarter; he considered that these cells were not chromaffin cells but, because of their intimate connections with nerves, were functionally connected with the sympathetic system. He proposed that they be named "hilus cells" or "Berger's hilus cells." Neumann¹⁸ also studied only ovaries and more or less agreed with Berger.

The most detailed morphological description of these cells in the testis is by Wieser⁷ who studied some 132 cases of all types and ages. Within each age group and each type of disease he found wide fluctuations and could come to no definite conclusion in respect to these features; the important fact was that point for point the "hilus" or "sympathicotropic" cells were identical with the regular interstitial cells.

Finally, Berger, in order to overcome the objection of de Winiwarter that Berger's cells were chromaffin cells, published in 1935¹⁹ a report of a testis from a newborn containing both chromaffin and "sympathicotropic" cells.

Extraparenchymal interstitial cells have been mentioned at various times, without any reference to their association with nerves, or to being any different from the ordinary interstitial cells. Berblinger,²⁰ Priesel,²¹ and Harms²² have mentioned the finding of groups of interstitial cells in the hilus of the testis, the tunica albuginea, or some location other than their usual one. Stieve⁸ in von Mollendorff's Handbuch states that in adults there are not infrequently found in the tunica albuginea small groups of interstitial cells, and that crystalloids may be found in these. Stieve⁸ and Brack²³ picture such cells.

In summary it may be said that within the last 15 years there has been described, chiefly by Berger but confirmed by a number of other investigators, the frequent presence of masses of cells in

the testicular and ovarian hili having intimate connection with the non-medullated nerves and having in both the ovary and the testis all the characteristics of Leydig cells with fluctuations paralleling the latter in the testis.

Personal Observations: Early in the study of the microscopic sections of this series of testes, and before becoming acquainted with the literature on the "sympathicotropic" cells, I was impressed by the same sort of findings as described by Berger and others. To anticipate, I may now say that my findings agree in all respects with those of the majority of writers on the subject, with the exception that these extraparenchymal interstitial cells occur not only in the hilar region of the testis but at any point within the tunica albuginea, whether distant from the hilus or not. (When referring to "within the tunica albuginea" I mean that the cell groups are entirely within the tunica and not merely invading its innermost laminae, as can be seen in almost any testis with numerous interstitial cells). Because they may be found at a distance from the hilus as often as not, the name "hilus cells" as proposed by Pawlowski is inappropriate. The name "sympathicotropic" under which they have heretofore usually been designated implies a functional status which is not proved. I therefore propose the name "extraparenchymal interstitial cells" or "extraparenchymal Leydig cells."

Of the 721 microscopically sectioned adult testes in this series, the tunica albuginea had been removed in 240 for ease in sectioning. In the remaining 481 there were groups of extraparenchymal interstitial cells in connection with nerves in the hilus or tunica albuginea in 109 testes, and the same cells not in connection with nerves in 85 testes; 45 of these 194 testes were duplicates, that is, cells both associated with and not associated with nerves were found in the same testis. In the 85 cases where masses of these cells were seen without immediately adjacent nerves it is believed that a sufficient number of adjoining sections would show their relation with nerves in the majority of cases, although probably not in all. For example, in some cases where the paraffin ribbon for one type of stain had not been taken immediately adjacent to that for another stain, but at some distance (say 100 μ) away, it was noted that one section would show the nervous connection and the other would not with the same group of cells.

Of the 149 testes that showed groups of cells in the hilus and tunica albuginea, either associated with nerves or unassociated, or both, 29 per cent showed ordinary interstitial cells of Grade 0, 42 per cent of Grade 1, and 24 per cent of Grade 2; this compares with 40, 43 and 13 per cent respectively for the entire series. I agree in general with the statements in the literature that when the ordinary or parenchymal interstitial cells are numerous, so are the extraparenchymal cells, although this is not always the case. I also agree as to the general similarity of the two in regard to morphology, pigmentation, crystals, and so on. Of the 149 testes with extraparenchymal interstitial cells, 21 showed pigment (Fig. 6) and 9 Reinke crystals (Fig. 7). Occasional multinucleated (2, 3, 4, and one with 8 nuclei) extraparenchymal interstitial cells (Fig. 4) were found. In my material they were, in agreement with the literature, found in all ages from 18 to 88 years.

One point must be remembered in regard to the stated frequencies of finding the extraparenchymal interstitial cells and that is that even in a midsagittal section of the testis with the tunica albuginea and hilus complete, the relative proportions of the latter that are sectioned as compared to those remaining unsectioned is very small, and therefore there must be numerous cases where these cells would be shown in serial sections but not in one or a few sections.

The size of the groups of extraparenchymal interstitial cells in my material varied from a few cells (which, of course, may have been the edge of a larger group) to masses which, including the enclosed nerve bundles, were 1.5 mm. in diameter (Fig. 5). The usual size of group found is shown in Figure 6. Very frequently, as mentioned in the literature, the cells were intraneural as well as perineural. There was no site of predilection for the cell groups. Previous writers have mentioned their occurrence chiefly in the hilus or rete testis, saying little about the tunica albuginea. In my material they were actually more frequent in the albuginea, because of the greater bulk of tissue, and relatively these cells could be found almost as frequently at any point within the albuginea, even directly opposite the hilus, as in the hilus. Previous writers have mentioned that these cells never occur actually within the epididymis, and neither did I find them there. Berger has found them along the spermatic cord as far as 6 cm. away from the testis.

These cells, again in agreement with the literature, are to be found in and around the intertubular as well as the paratesticular nerves, but with greater difficulty (except just inside the tunica albuginea, where they can readily be seen). There are two reasons for this: the nerves within the testis are smaller and because of the multiplicity of tissues in the interstitium are more difficult to observe. However, this perineural relationship can be observed within the testis, especially after tubular atrophy has taken place and the nerves stand out more sharply.

SUMMARY

1. In a study of the microscopic sections of 721 testes from a series of 470 autopsies on males 18 years of age or more, giant interstitial (Leydig) cells having from 4 to 30 (usually 8 or 10) nuclei were found in 85 testes. They were found at all ages and in all sorts of general disease conditions. They do not appear to have been previously described, although their existence has been hinted at.

2. The observations of Berger and others on the "sympathicotrophic" or "hilus" cells have been confirmed and extended. It is generally agreed that these cells in the testis are identical with the ordinary interstitial or Leydig cells. I have proposed the name "extraparenchymal interstitial cells" or "extraparenchymal Leydig cells" as best describing them.

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DESCRIPTION OF PLATES

PLATE 154

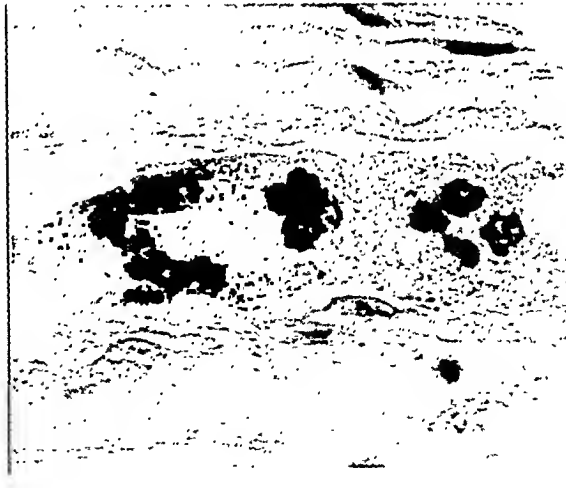
- FIG. 1. Case 296. 72 years; cerebral thrombosis. Group of giant interstitial cells among mononuclear interstitial cells. The pigment zone and clear center of the giant cells are well shown, as is the similarity of their nuclei to those of the ordinary interstitial cells. Hematoxylin-eosin. $\times 325$.
- FIG. 2. Case 194. 60 years; cerebral hemorrhage. Giant interstitial cell with 21 nuclei; one of the nuclei is more vesicular than the others. To the right are three mononuclear interstitial cells. Hematoxylin-eosin. $\times 650$.
- FIG. 3. Case 231. 65 years; coronary sclerosis. Giant interstitial cell with 14 nuclei. To the right are several mononuclear interstitial cells. Hematoxylin-eosin. $\times 650$.



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Nelson

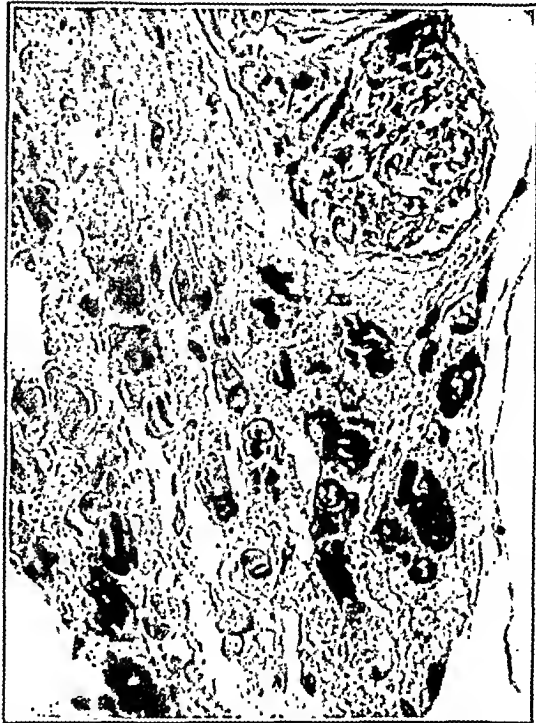
Giant Interstitial Cells of Testis

PLATE 155

- FIG. 4. Case 231. Giant interstitial cell with 8 nuclei in tunica albuginea of the same testis as shown in Figure 3. Hematoxylin-eosin. $\times 800$.
- FIG. 5. Case 499. 82 years; hypertension. Large mass of extraparenchymal interstitial cells surrounding nerve fiber bundles in hilar region. Hematoxylin-eosin. $\times 60$.
- FIG. 6. Case 335. 66 years; polycystic kidneys. Small mass of pigmented extraparenchymal interstitial cells around a nerve in the tunica albuginea. This is the usual size of group seen. Hematoxylin-eosin. $\times 330$.
- FIG. 7. Case 547. 77 years; exfoliative dermatitis. Group of extraparenchymal interstitial cells in the tunica albuginea, containing numerous Reinke crystals and in association with a nerve. Azocarmine. $\times 465$.



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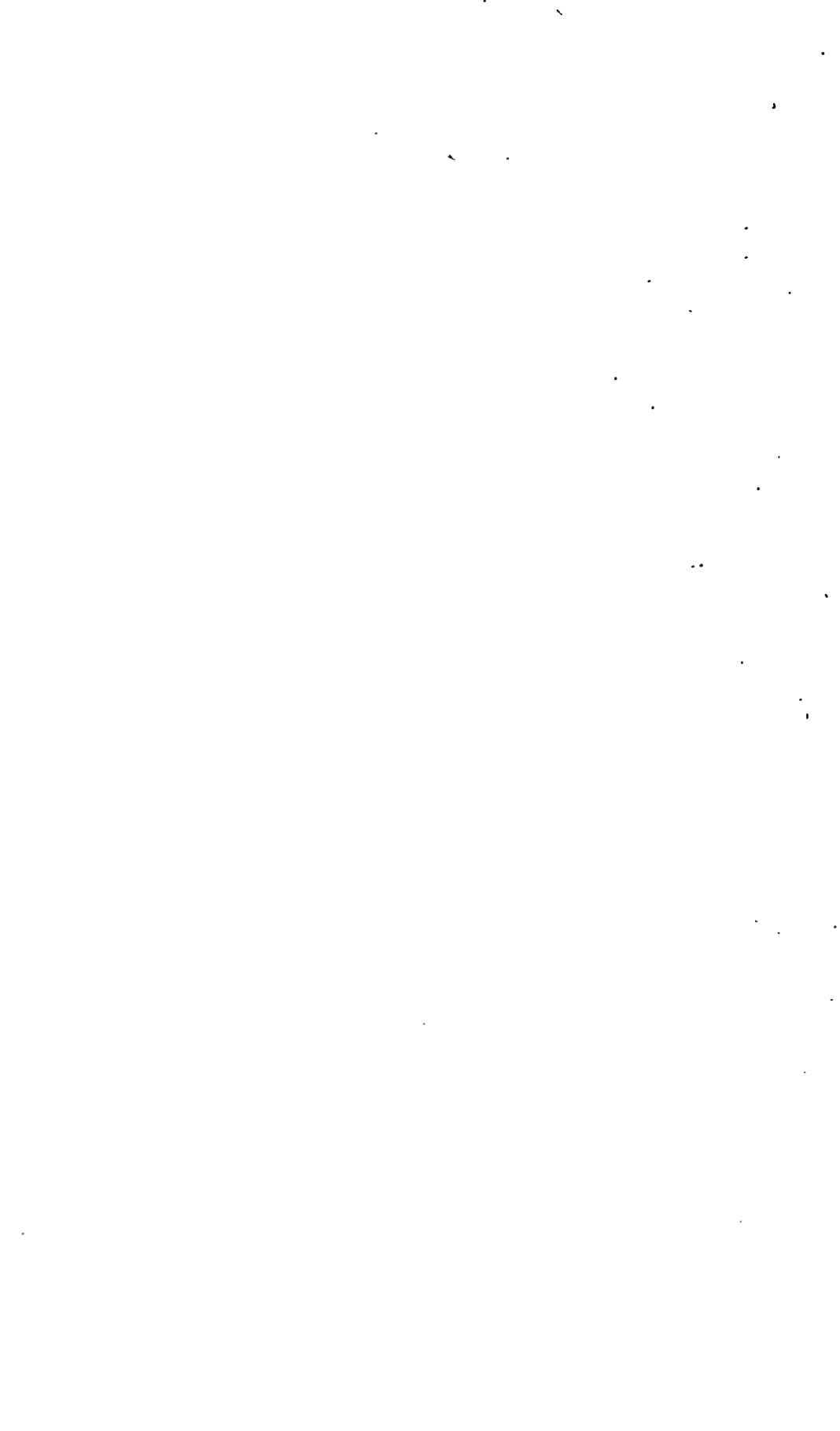


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THE EFFECT OF ASCORBIC ACID DEFICIENCY ON ENAMEL FORMATION IN THE TEETH OF GUINEA PIGS *

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The effect on enamel formation of diets deficient in ascorbic acid has received relatively little attention. Changes in this tissue are not mentioned in the classical work of Höjer¹ or of Wolbach and Howe,² although alterations in the dentin and dental pulp are described in detail. Kotányi³ reported alterations of both the enamel-forming cells and the enamel itself in the teeth of scorbutic guinea pigs. Recently Fish and Harris⁴ have emphasized that the changes in the enamel-forming cells in "full scurvy" are characteristic of the scorbutic process, although they were unable to demonstrate changes in these cells in "subscurvy." Their conclusion that "the failure of normal enamel formation, to which we find vitamin C deficiency gives rise, may be of significance in the causation of human caries" has been widely quoted.

Since the ameloblasts are of ectodermal origin, demonstration that a deficiency of ascorbic acid has a primary effect on these cells would constitute an exception to the findings of Wolbach^{2,5} that lesions caused by this deficiency are a consequence of the inability of certain cells of mesenchymal origin to produce and maintain normal intercellular substances and that other effects are secondary. Furthermore, evidence purporting to bear on the disputed etiology of dental caries should be thoroughly scrutinized.

MATERIAL AND METHODS

Ground and decalcified sections of the teeth of a large number of guinea pigs were examined.⁶ Sections in cross and longitudinal planes of both incisor and molar teeth were stained routinely with hematoxylin and eosin. Mallory's aniline blue collagen stain and the phosphotungstic acid hematoxylin method were also fre-

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quently employed, and many sections were prepared by the Gömöri technique.⁷

FINDINGS

Enamel deposition in the continually growing molar and incisor teeth of the guinea pig commences shortly after the first dentin is deposited. Enamel is laid down peripherally to the dentin until it reaches its maximum thickness at a point about one-third of the distance from the formative end (apex) to the end in function with its opponents of the opposite jaw (incisal or occlusal surface). At this point the enamel organ of the molar teeth is invaded by mesenchymal cells. Over the enamel surfaces surrounded by alveolar bone these cells deposit blocks of cementum which serve as attachments for the suspending fibers of the periodontium. Between the folds of the molar teeth a peculiar cartilage cement is deposited upon the enamel surfaces.

In the incisor teeth the enamel-forming cells become reduced in height after enamel deposition ceases, although they remain distinguishable until near the gingival crevice where they become merged with the cells of the oral epithelium. The cementum covers only a small part of the enamel surface on the buccal and lingual sides of the tooth. As has been previously pointed out,⁸ the enamel covered area of the incisor teeth plays little part in the suspension of the teeth from the surrounding bone.

Enamel, as first deposited in both the molar and the incisor teeth, resists decalcification and stains deeply with basic dyes (Fig. 1). As the enamel approaches maturity it becomes partially decalcified when treated with 5 per cent nitric acid. The extreme complexity of the pattern made by the interwoven enamel rods becomes evident at this stage (Fig. 2). The outer border of the immature enamel remains unstained by the silver nitrate of the Gömöri technique (Fig. 3).

Careful examination of both ground and decalcified sections reveals that, contrary to Santoné,⁹ many of the dentinal fibrils continue into the enamel. When stained by Mallory's aniline blue collagen stain the fibrils are colored a brilliant red. They are very fine, form a plexus in the outer part of the dentin and then angulate sharply to follow the course of the enamel rods so that they are difficult to trace.

Normally the width of the enamel is less than the width of the underlying dentin. The ratio of enamel to dentin is approximately 3:4.

Animals maintained on diets completely deficient in ascorbic acid show marked retardation of dentin deposition while enamel is deposited at approximately the normal rate. The dentin-forming cells are characteristically atrophic, while the enamel-forming cells appear normal (Fig. 4). The thickness of the enamel is accordingly several times greater than that of the corresponding dentin. Ratios of enamel to dentin vary from 3:1 to 4:1 (Figs. 4 and 5). When animals are maintained on diets completely deficient in ascorbic acid but supplemented with small amounts of this substance administered daily, the width of the enamel as compared to the dentin approaches normal as the dosage is increased (Figs. 6 and 7). Animals receiving 2 mg. or more daily have normal enamel and dentin. In both complete and partial deficiencies the enamel may show areas of hypoplastic structure. Such areas usually are associated with hemorrhage in the overlying tissues and with disruption and atrophy of the enamel-forming cells (Figs. 8, 9 and 10).

The periodontal tissues over the cementum covered parts of the tooth also show areas of hemorrhage and a diminution in the number of collagen fibers (Figs. 11, 13 and 14). Such regions appear cellular in comparison with the periodontal tissue of the normal animal (Fig. 15). Nevertheless, mitotic figures among these fibroblasts in ascorbic acid deficient animals are rare. When ascorbic acid is administered repair may be very rapid as indicated by numerous mitoses (Fig. 12).

DISCUSSION

Loosening of the teeth has long been recognized as a cardinal sign of scurvy, both in man and in the experimental animal. This phenomenon occurs as the result of inability of the fibroblasts of the periodontium to form the collagen fibers by which the teeth are suspended from the surrounding bone and the corresponding inability of the osteoblasts and cementoblasts to form normal matrices for the attachment of these fibers.

Enamel formation is normally carried on in a protected environment. In the teeth of man the enamel is completely formed before

eruption into the oral cavity occurs. The deciduous teeth are so placed as to give added protection to their permanent successors while the latter are developing. It is a well known clinical observation that the premature extraction of the deciduous teeth may so injure the enamel-forming cells of the underlying permanent tooth that an area of hypoplastic enamel results.

Enamel formation in the constantly growing teeth of the guinea pig occurs while the teeth are in function. The mechanisms by which the forces of mastication are absorbed and dissipated in the part of the periodontium nearest the occlusal surface of the teeth while the formative part of the periodontium is protected from trauma has been the subject of a recent report.¹⁰ It was concluded that a protected environment for the formation of enamel exists and that the collagenous suspending fibers play an important part in its maintenance. Failure of collagen fiber formation, the characteristic phenomenon of ascorbic acid deficiency, allows the transmission of excess forces to the formative part of the periodontium, adequately accounting for the areas of defective enamel formation described. Thus, enamel hypoplasia is a secondary rather than a primary consequence of the deficiency and corresponds to the hypoplasias of human teeth produced by trauma to the enamel organ.

Since the enamel of human teeth is formed before the teeth erupt into the oral cavity, the tooth germs are not exposed to masticatory forces while enamel is being deposited. Study of the tooth germ in infantile scurvy¹¹ and examination of the permanent teeth of a limited number of patients who recovered from infantile scurvy indicate that enamel hypoplasia does not result from ascorbic acid deficiency during the period of tooth formation. As in the guinea pig, though to a much less degree, dentin formation may be retarded so that the enamel may be of greater width than the underlying dentin, leading to the formation of a dwarfed crown covered with normal enamel.

Dental caries of the exposed dentin and cementum occurs in both incisor and molar teeth of the guinea pig.¹² However, no correlation with deficiency of the diet has been found. Caries of the normal or hypoplastic enamel has not been observed.

The clinical reports of Aschoff and Koch¹³ and of Westin¹⁴ indicate that a possible immunity rather than an increased suscep-

tibility to dental caries occurs in ascorbic acid deficiency in human beings.

From the evidence at present available, dental caries in man and in the guinea pig does not appear to be due to a deficiency of ascorbic acid in the diet.

SUMMARY AND CONCLUSIONS

1. Enamel formation is not primarily affected by ascorbic acid deficiency. Areas of hypoplastic enamel formation observed in both complete and partial ascorbic acid deficiency are adequately accounted for by the failure of collagen fibers and bone matrix to form in the periodontal tissues.

2. Enamel-dentin thickness ratios greater than 1 in ascorbic acid deficiency result from continuation of enamel formation at approximately the normal rate while dentin formation is retarded.

3. Satisfactory evidence of the relation between ascorbic acid deficiency and dental caries has not been established.

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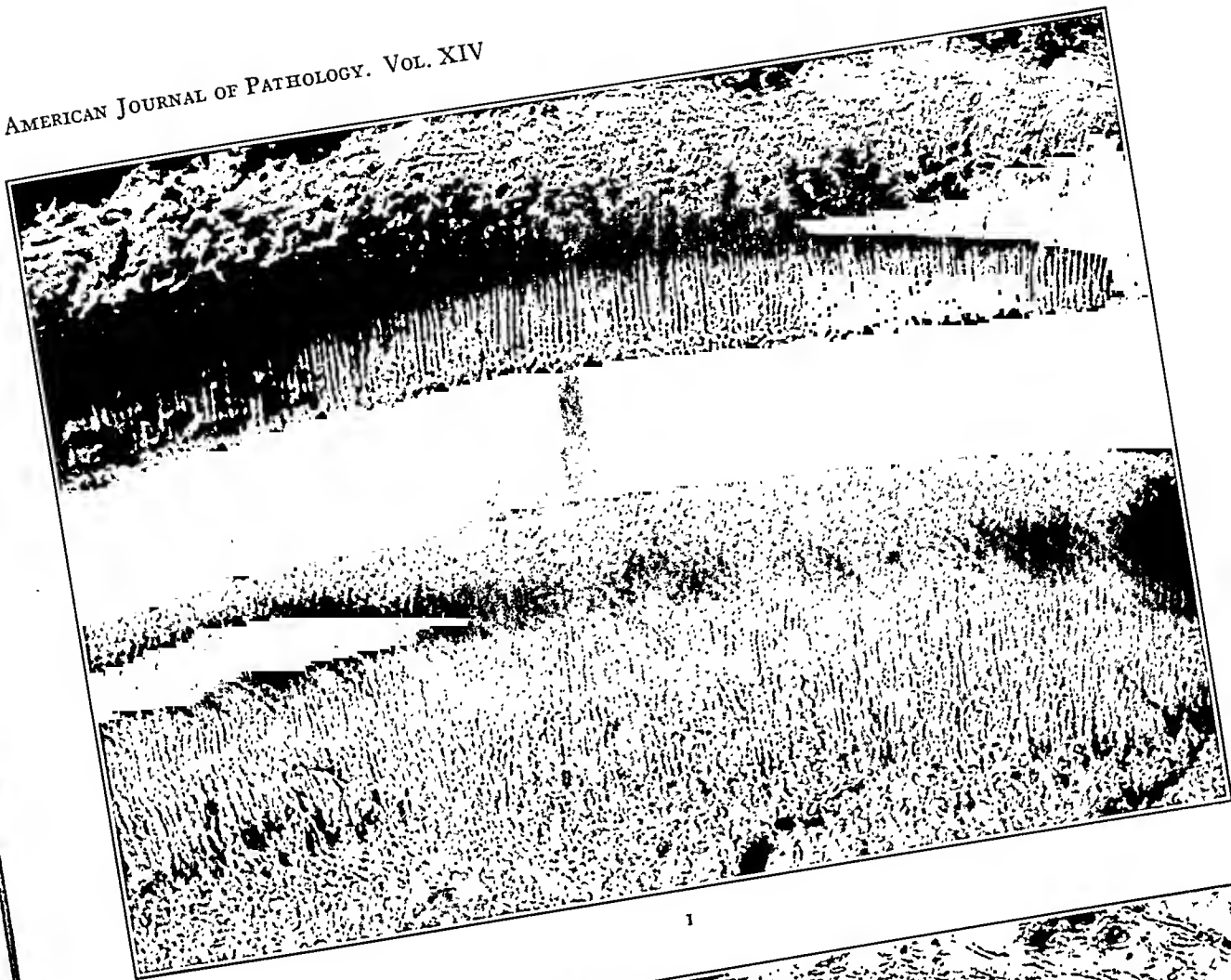
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DESCRIPTION OF PLATES

PLATE 156

FIG. 1. Guinea pig 76. Normal control animal. Early stage of enamel and dentin formation. Enamel-dentin ratio 3:4. Structures from above downward are vascular connective tissue, enamel organ, enamel, dentin, odontoblasts, and cells of the dental pulp. $\times 270$.

FIG. 2. Guinea pig 77. Normal control animal. Later stage of enamel and dentin formation. Enamel-dentin ratio 3:4. $\times 270$.



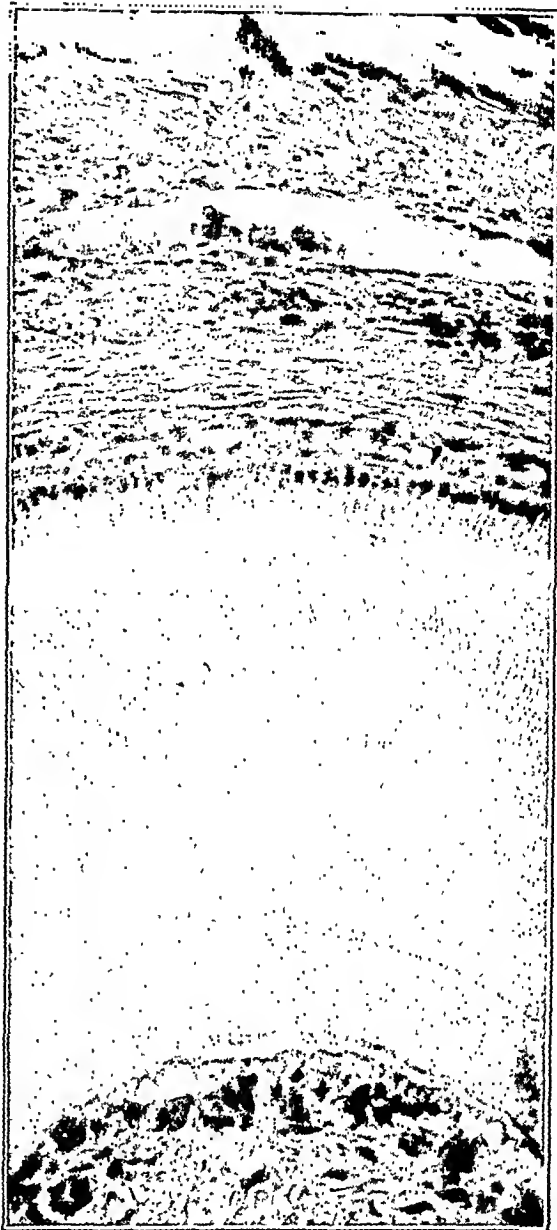
Effect of Ascorbic Acid Deficiency

PLATE 157

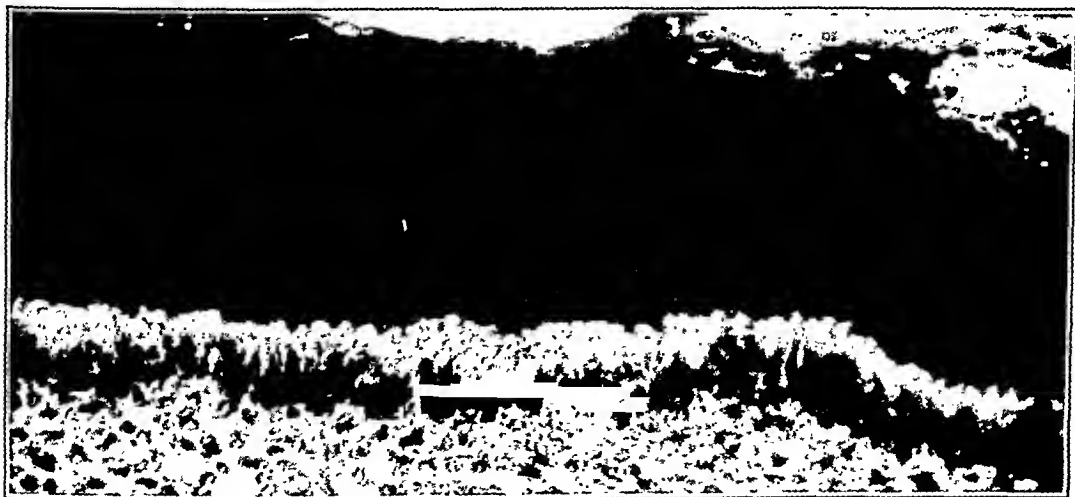
- FIG. 3. Guinea pig 93, 109 days on the basal diet plus greens (positive control). The section was prepared by the Gömöri technique and shows the zone immediately beneath the ameloblasts unstained by silver nitrate. Structures in same order as in Figure 1. $\times 300$.
- FIG. 4. Guinea pig 152, 28 days on a diet free of ascorbic acid. The enamel-forming cells and the enamel itself are normal, while the odontoblasts and the dentin show the extreme atrophy characteristic of this deficiency. Structures from above downward are the alveolar bone, loose fibrous tissue with large blood vessels, enamel organ and its ameloblasts, enamel, dentin, odontoblasts, and cells of the dental pulp. $\times 300$.
- FIG. 5. Guinea pig 113. Basal diet alone for 28 days. Early stage of enamel and dentin formation. Dentin formation has been retarded while enamel deposition has taken place normally, resulting in a relatively wide enamel layer. Enamel-dentin ratio 3:1. $\times 300$.



3



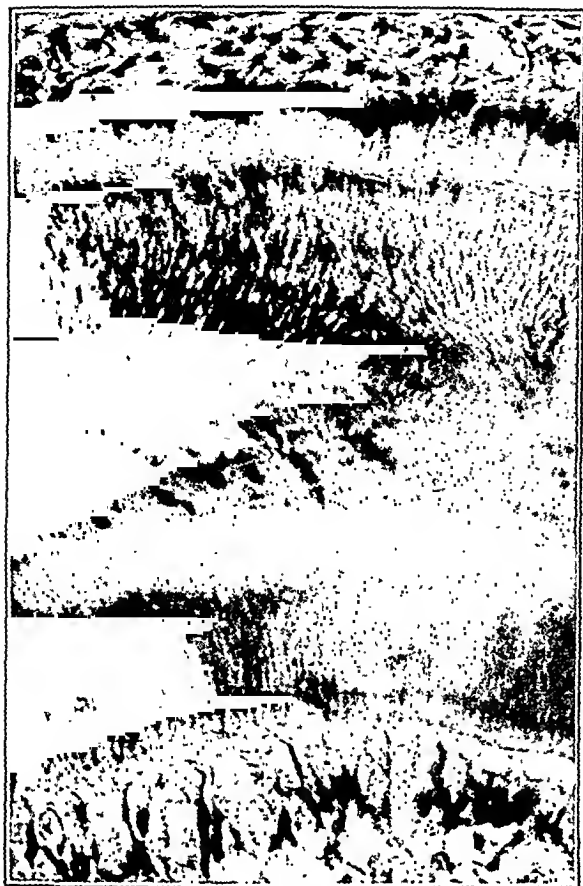
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PLATE 158

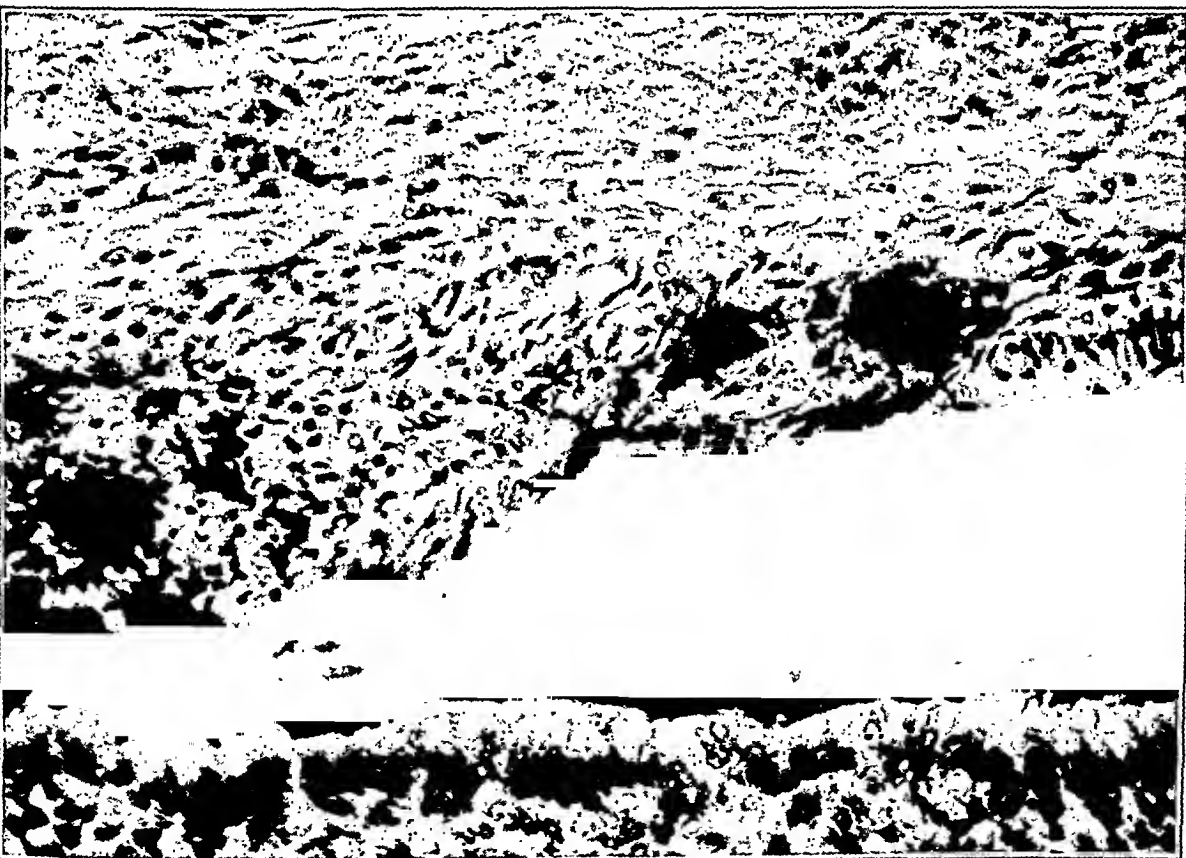
- FIG. 6. Guinea pig 66. Basal diet supplemented by 0.5 mg. of ascorbic acid daily for 182 days. Enamel-dentin ratio 2:1. $\times 300$.
- FIG. 7. Guinea pig 140. Basal diet supplemented by 0.3 mg. of ascorbic acid daily for 329 days. Enamel-dentin ratio 4:1. $\times 300$.
- FIG. 8. Guinea pig 113. Basal diet for 28 days. Hypoplastic enamel formation. Atrophy of ameloblasts in region of irregular enamel deposition and hemorrhage in the overlying tissues. $\times 300$.



6



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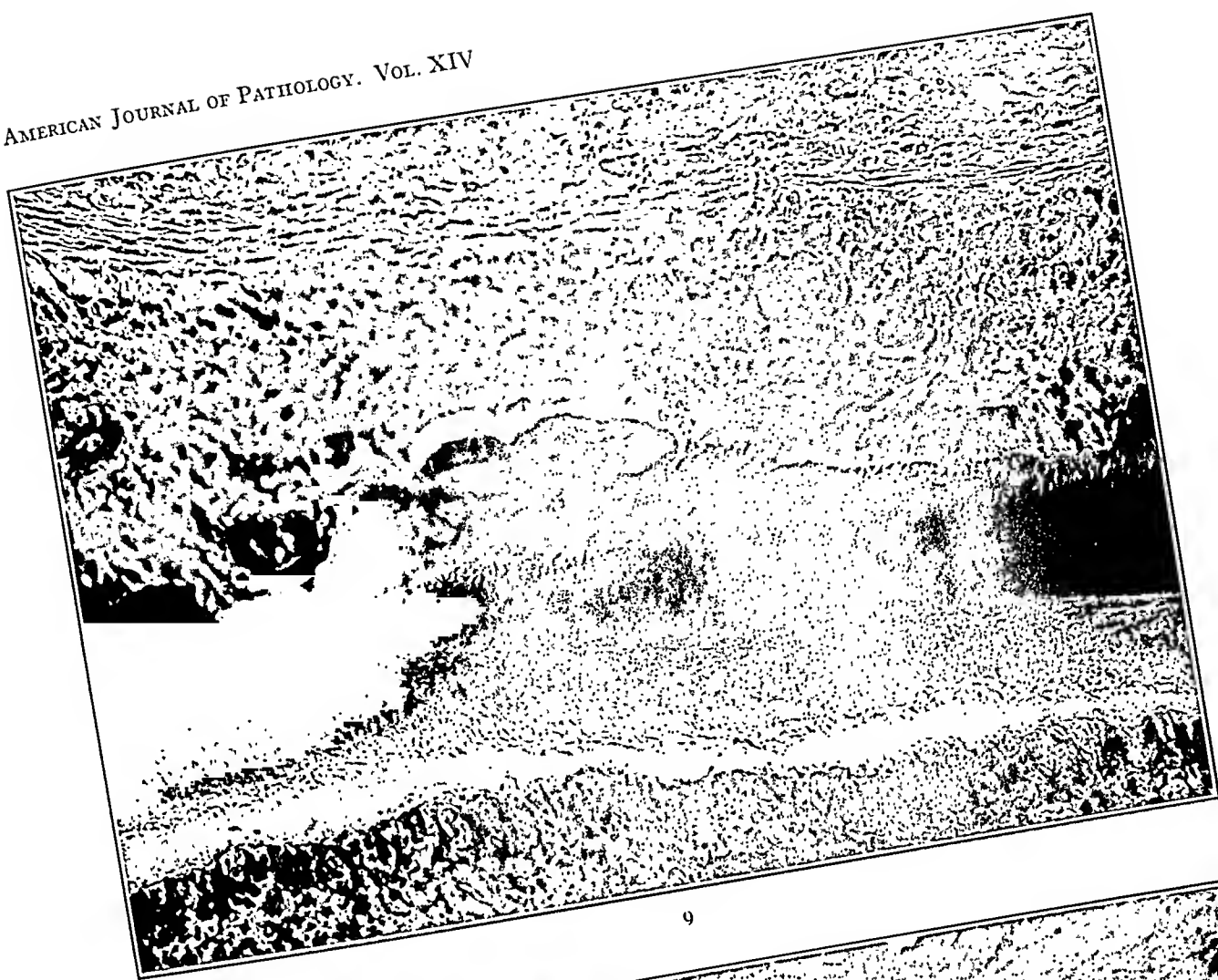


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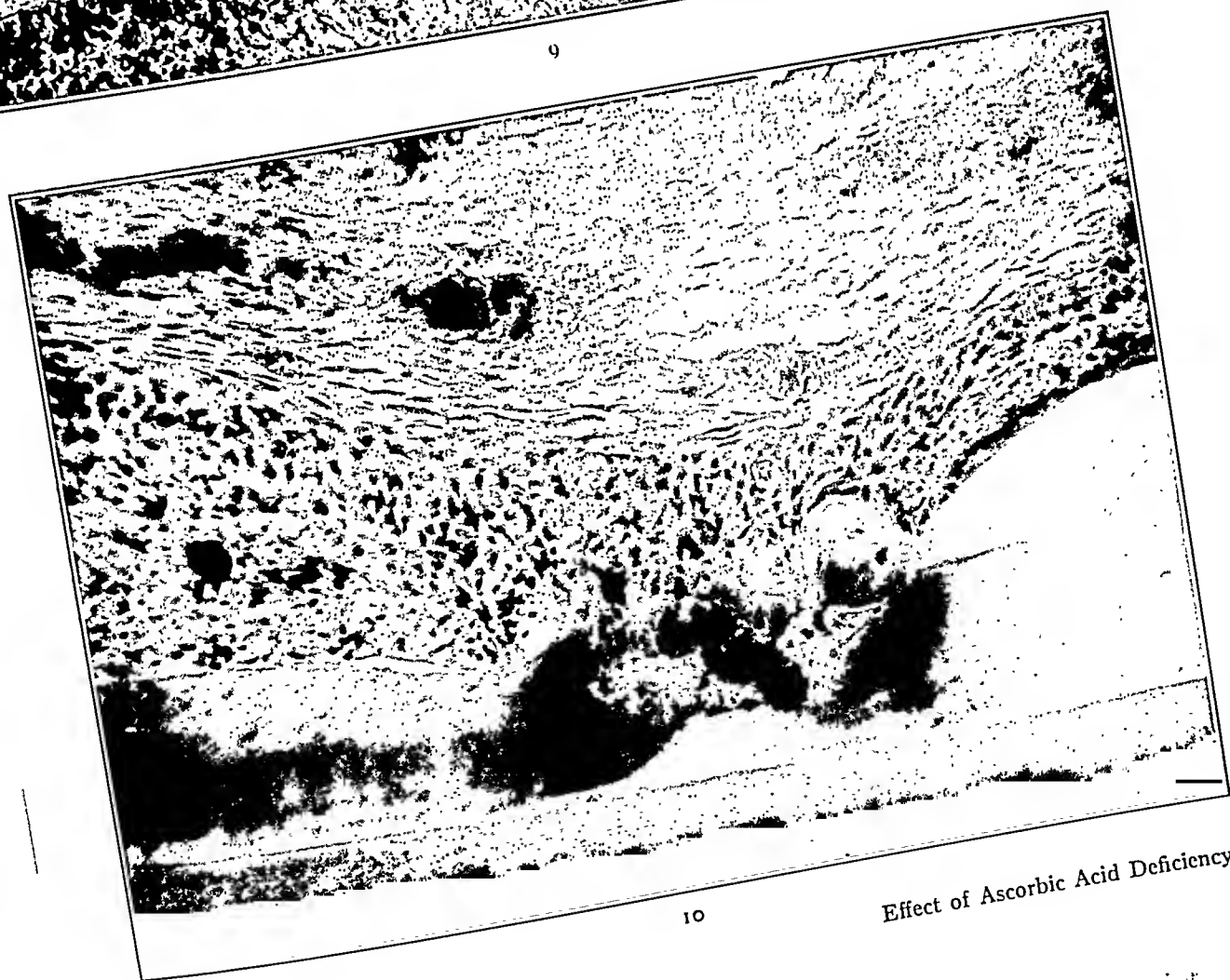
PLATE 159

FIG. 9. Guinea pig 152. Basal diet for 28 days. Area of hypoplastic enamel formation. $\times 300$.

FIG. 10. Guinea pig 18. Basal diet supplemented by 1 mg. of ascorbic acid daily for 120 days. Area of hypoplastic enamel formation with atrophy of the ameloblasts and hemorrhage in the overlying tissues. This is a relatively rare finding in animals receiving more than 0.5 mg. of ascorbic acid daily. The enamel-dentin ratio elsewhere than in this area was normal (3:4). $\times 300$.



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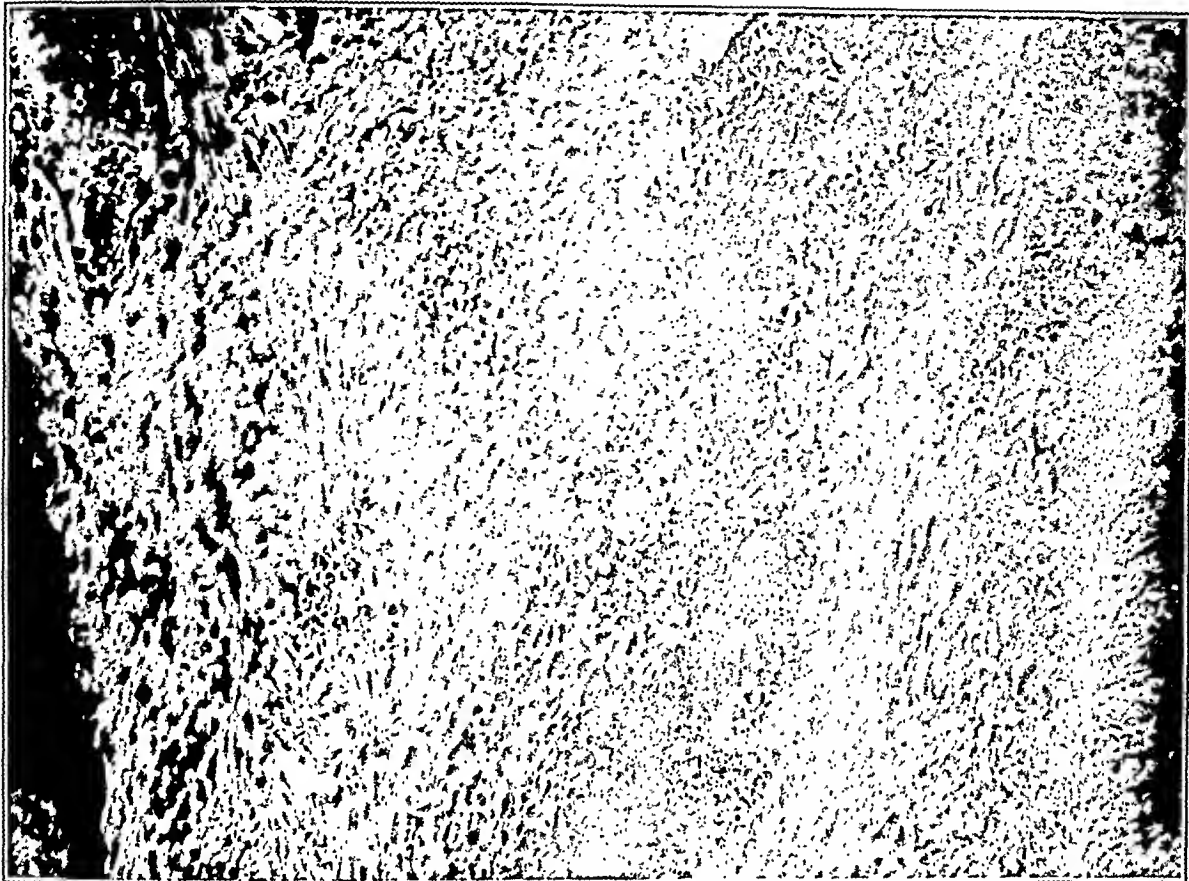


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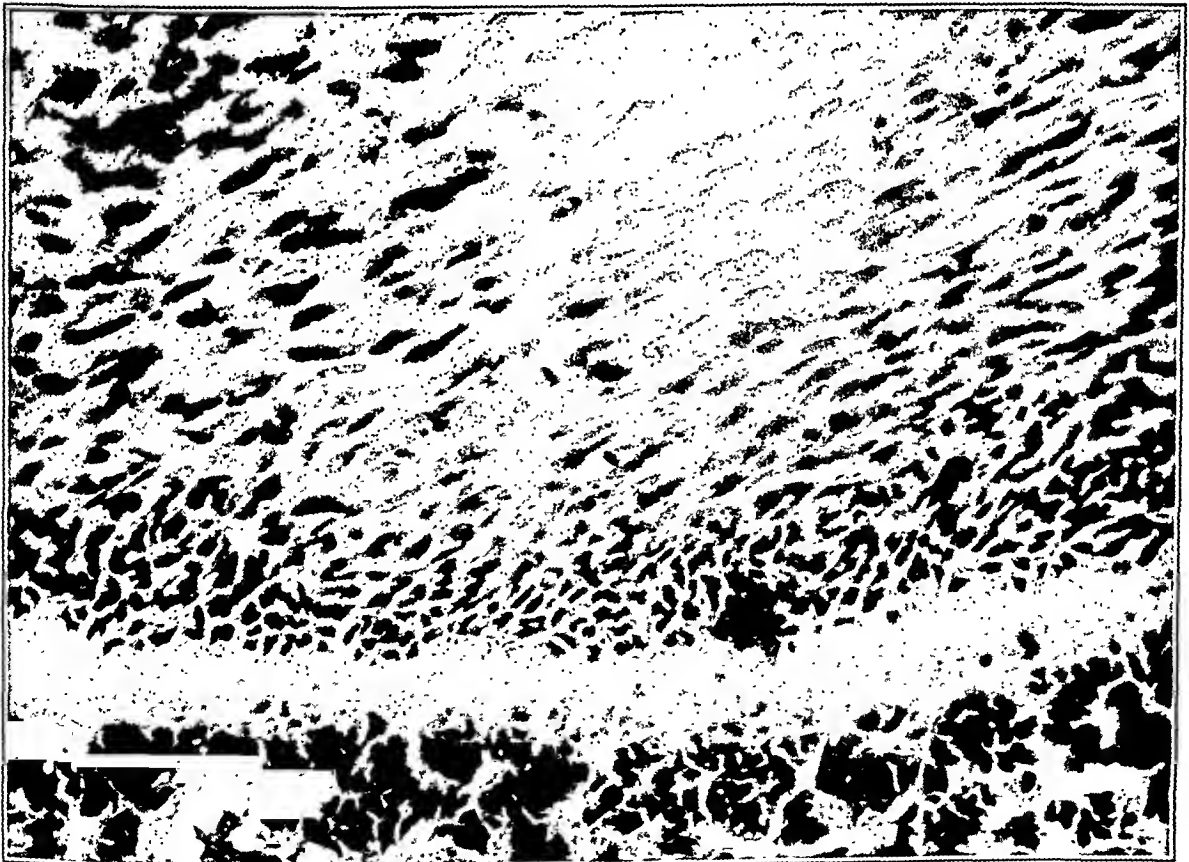
Effect of Ascorbic Acid Deficiency

PLATE 160

- FIG. 11. Guinea pig 152. Hemorrhage in the peridental tissues on the cementum side of the tooth opposite the hypoplastic area is shown in Figure 9. $\times 300$.
- FIG. 12. Guinea pig 116. Basal diet for 35 days, 100 mg. of ascorbic acid by mouth in 2 doses, killed 48 hours after the 1st dose. Active repair in the fibrous tissue overlying the cementum. Note the mitotic figures. $\times 300$.



11



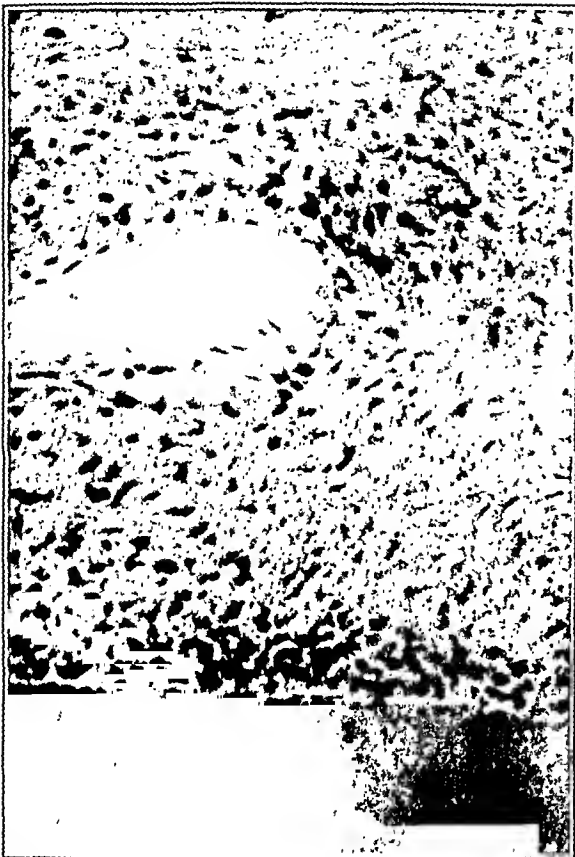
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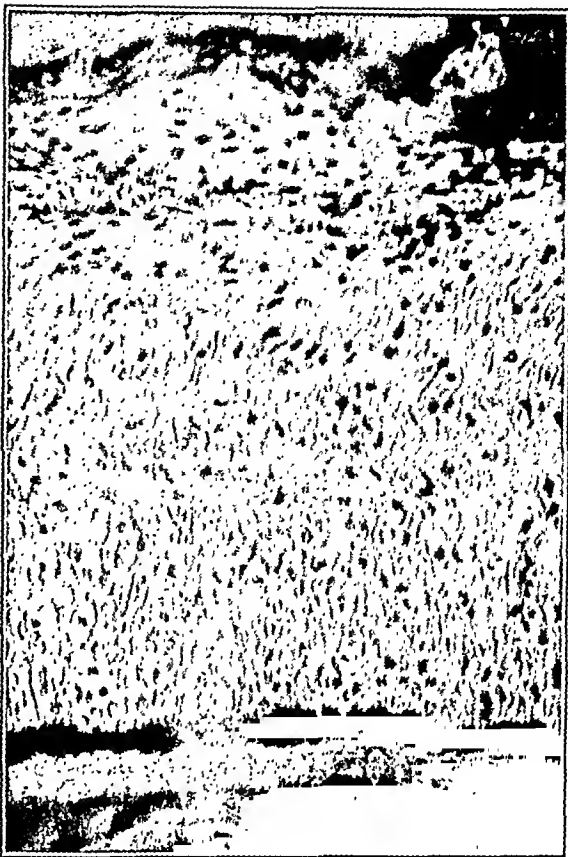
FIG. 13. Guinea pig 31. Basal diet for 27 days. Fibrous tissue connects the overlying alveolar bone with the cementum covered surface of the tooth. The cells are atrophic and collagen fibers are lacking or represented by a granular intercellular material. $\times 300$.

FIG. 14. Guinea pig 141. Basal diet supplemented by 0.3 mg. of ascorbic acid daily for 252 days. Many of the fibroblasts show vacuoles and the cementum is hyperplastic in response to loosening of the tooth. $\times 300$.

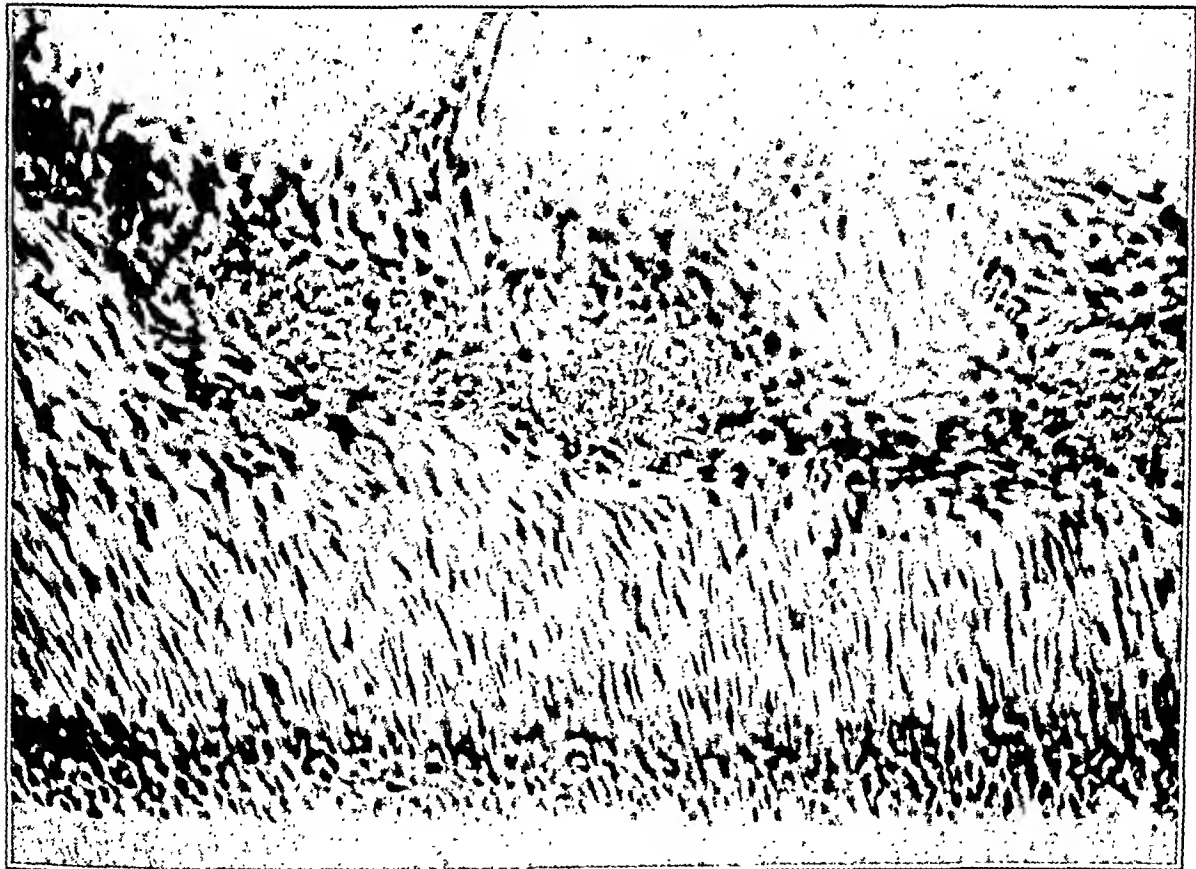
FIG. 15. Guinea pig 94. Basal diet supplemented by 5 mg. of ascorbic acid daily for 121 days (positive control). The bone, fibrous tissue, cementum and dentin are normal in structure. $\times 300$.



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CONCLUSIONS

The following observations were made during the study of 16 tubes in which judged carcinomatous implants on the mucosa, secondary to carcinoma of the ovary, were found.

1. Changes in the tubal mucosa favorable for the implantation of cancer cells on its surface, such as: (*a*) local proliferations of the cells of the subepithelial tissues of the mucosa associated with a partial or a complete loss of the overlying epithelium; and (*b*) granulation tissue of various types on the surface of the mucosa, usually in the form of sessile or pedunculated polypoid outgrowths in some of which newly formed lymph vessels were detected.

2. Cancer cells splinted by fibrin on areas of the mucosa with a local reaction of their subepithelial tissues and also cancer cells attached to these areas without the presence of fibrin. Cancer cells attached to or becoming embedded in the polypoid granulation tissue just described.

3. Carcinoma replacing portions of the tubal epithelium as though grafted in it. Other neoplasms consisting of carcinoma either growing on or embedded in organized newly formed tissue attached to the surface of the mucosa, usually in the form of sessile or pedunculated polypoid tumors.

4. All stages in the development of each of these three types of mucosal implantation metastases.

5. Sometimes implanted carcinoma dies. In other instances it grows slowly and apparently remains localized for a long time. Again conditions are found indicating that implanted carcinoma has invaded the mucosal tissues beneath it including the lymph vessels just as a primary carcinoma invades and spreads.

6. The pathogenesis, structure, form and life history of carcinomatous implants of ovarian origin on the tubal mucosa are the same as the pathogenesis, structure, form and life history of similar implants on the peritoneal serosa.

NOTE: The efficiency of the laboratory work for this paper is, in large measure, due to the technical skill and the interest of Miss Helen Buchan. The illustrations of the gross specimens were made by Mrs. M. R. Marden and the microphotographs by Mr. James A. Glenn. These I thank for their interest and cooperation.

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DESCRIPTION OF PLATES

PLATE 78

FIG. 1. Microphotograph of a longitudinal section of the distal portion of the ampulla of a normal appearing tube. The patient, A. H. No. 26207, nulliparous and aged 43 years, had had the appendix, the entire uterus and both tubes and ovaries removed for carcinoma of the body of the uterus. The hysterectomy had been preceded by the intrauterine application of radium followed by deep X-rays. The tube had been incised longitudinally before placing it in formalin in order to avoid the compression of its mucosa incident to the fixation of an intact tube. A large mucosal fold and many smaller folds, beneath it, are shown in this microphotograph. This section includes only a small portion of the base of the large fold at the left. Note the large lymph vessel running longitudinally in the large fold. Since it is a true capillary without valves, cancer cells gaining access to its lumen may easily spread in all directions in the lymph plexus of the fold, even into the fimbrial mucosal fold which is a continuation of this ampullar fold. Note also a similar arrangement of the lymphatics in the smaller folds. A reading glass is of great value in studying all of the microphotographs. $\times 10$.

FIG. 2. The pattern of the lymphatics of the mucosa of the ampulla of a normal appearing tube as seen in cross section. The patient, A. H. No. 28, parous and aged 40 years, had had the appendix, the entire uterus and one tube and ovary removed for an adherent retroflexed uterus associated with peritoneal endometriosis. The tube had been incised longitudinally before placing it in formalin. Irrespective of the size of the mucosal folds the general pattern of their lymphatics is the same. The lymphatics in the folds empty into vessels at the base of the folds which are continuous with vessels between the folds. Thus the lymphatics of one mucosal fold are united with those of adjacent folds. The lymphatics of the secondary mucosal folds empty at their base into the vessels of the primary folds just as the lymph vessels of the primary folds drain into the vessels at their base. Note that while the lymphatics tend to be centrally situated in the folds they may be, in places, close to the epithelium. A portion of a large mucosal fold appears at the right. A longitudinal section through such a fold might not include its base, as occurred in the preceding section. $\times 25$.

FIG. 3. Microphotograph similar to the preceding one. The patient, A. H. No. 699, parous and aged 40 years, had had the appendix, the entire uterus and one tube and ovary removed for a uterine leiomyoma. The pattern of the lymphatics of the mucosa of the ampulla, as seen in cross section, is the same as that shown in the preceding microphotograph. A study of the lymphatics of the mucosa of the ampulla of many tubes has convinced me that in a general way this pattern is constant with minor variations according to the size and complexity of each mucosal fold. $\times 25$.



1



2



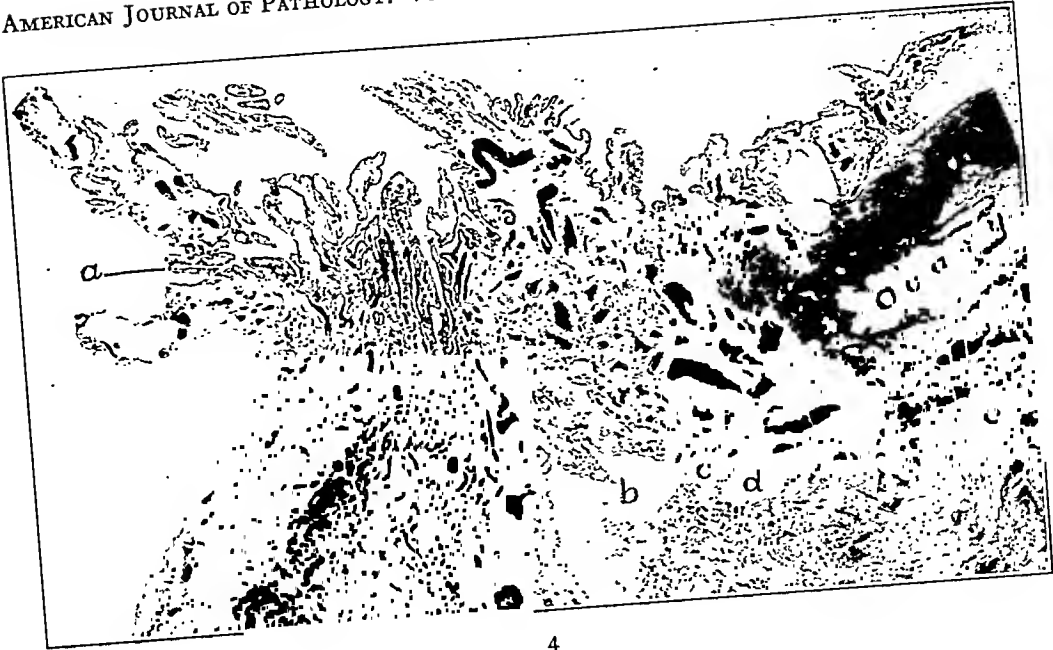
3

PLATE 79

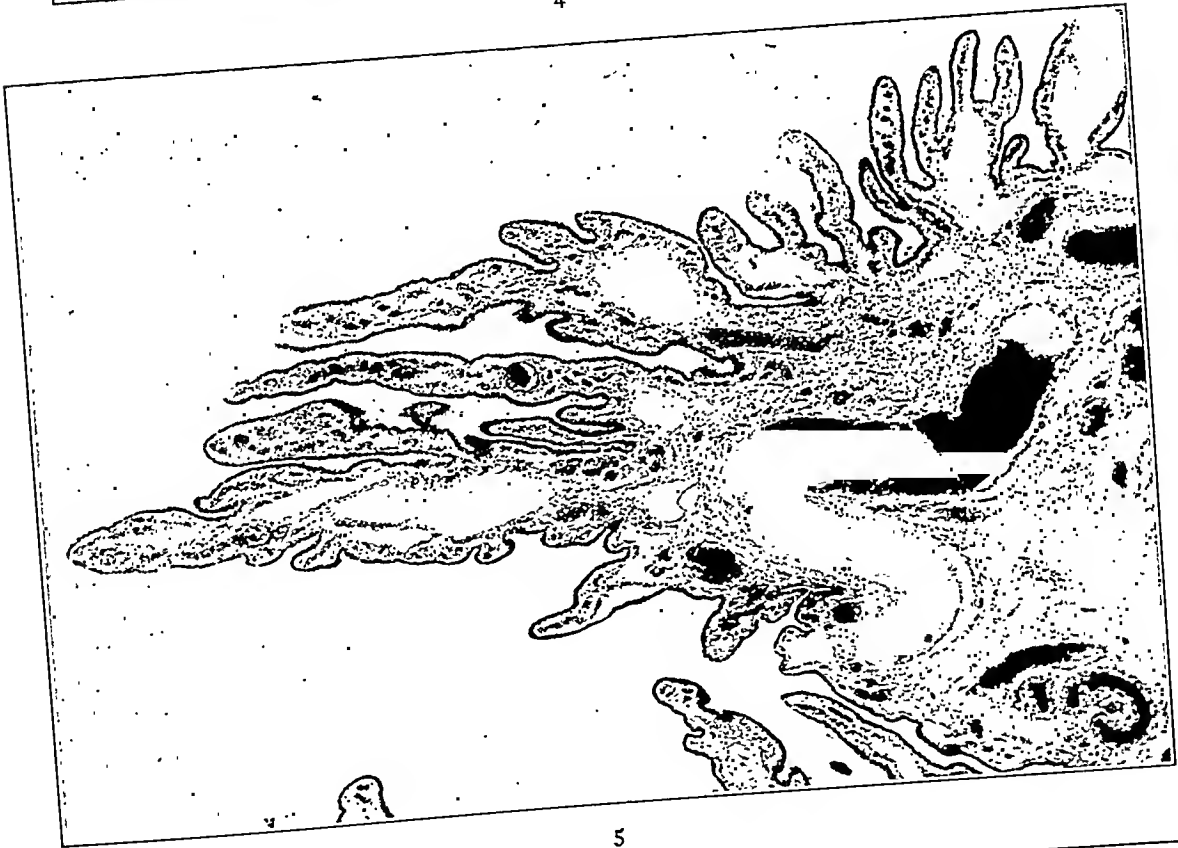
FIG. 4. Longitudinal section of the distal portion of a normal appearing tube including its fimbriae proper, the mesosalpinx with its ovarian fimbriae, and the tubal pole of the ovary at the right. The patient, A. H. No. 9339-31, parous and aged 47 years, had had the entire uterus and one tube and ovary removed for a uterine leiomyoma. After removing the uterus and before releasing the clamp on the ovarian vessels these vessels were ligated. A ligature was also placed about the tube, broad ligament and utero-ovarian ligament, near the uterus. The tube and ovary were then severed from the uterus and immediately placed in formalin. The veins thus filled with blood, as with an injection mass, appear black in the microphotograph. Their distribution suggests an intimate relationship between venous outlets of the tubal pole of the ovary and those of the fimbriae and the mucosa of the distal portion of the tube. The large irregular space "b" arose from an incomplete section of the mesosalpinx. I believe that the spaces "c," "d" and "e" are the lumens of large lymph vessels. Under higher magnification occasional constricted lymph vessels can be seen in the compressed folds of the mucosa of the ampulla and dilated lymph vessels, in many of the folds of the fimbrial mucosa. The fimbrial mucosa is but a continuation of the mucosa of the ampulla beyond the ostium of the tube. The lymphatics in the mucosa in the two situations are continuous (see Fig. 9 of previous paper¹¹). Carcinoma gaining access to the lymphatics of the fimbrial mucosa could easily spread to those of the ampullar mucosa and *vice versa*. This might account for the occurrence of carcinoma in both situations in many instances. $\times 5$.

FIG. 5. Higher magnification of a portion of the fimbrial mucosa to the right of the abdominal ostium of the tube shown in the preceding microphotograph. The pattern of the lymphatics in the mucosal folds of the fimbriae and ampulla shown in Figures 2 and 3 is the same. $\times 25$.

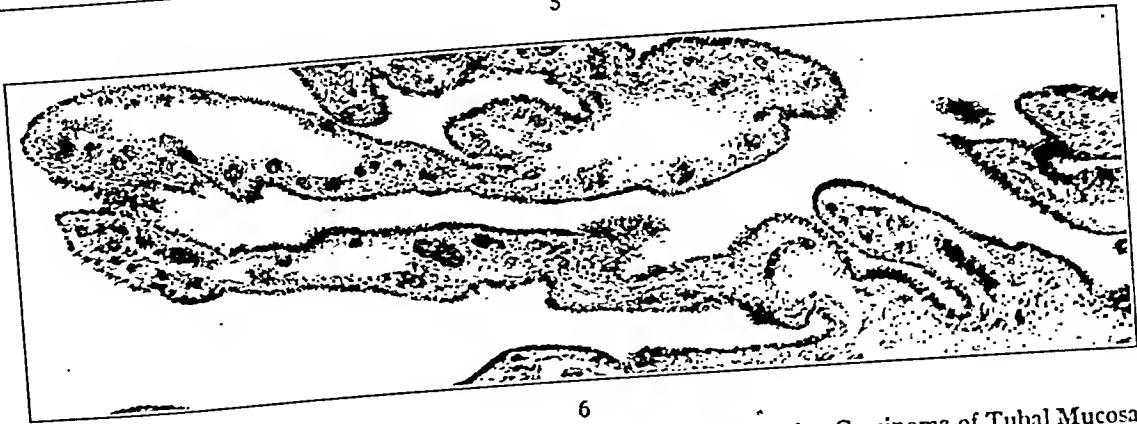
FIG. 6. Higher magnification of a portion of the mucosal folds of the fimbriae indicated by "a" in Figure 4. Note that while the lymph vessels in the mucosal folds are for the most part more centrally located than the blood vessels, in places they are separated from the peritoneal cavity by only a very thin layer of tissue covered by epithelium. This proximity of the lymph vessels to the surface of the mucosa suggests not only their accessibility to carcinoma of the tubal mucosa, but also their availability for the origin of newly formed lymph vessels as well as newly formed blood vessels in granulation tissue which, in some instances, arises on the surface of the mucosa (see Figs. 71, 95 and 96). $\times 54$.



4



5



6

Implantation Carcinoma of Tubal Mucosa

PLATE 80

FIG. 7. Longitudinal section of a portion of the fimbrial mucosa of a normal appearing tube. The patient, A. H. No. 28737, parous and aged 42 years, had had the entire uterus and one tube and ovary removed for a uterine leiomyoma. The veins partially or completely filled with blood are evident. The dilated lymphatics, by contrast, are just as striking as the veins. Note the central location of the large lymph vessels in the mucosal folds and the situation of the blood vessels between them and the surface of the folds. $\times 25$.

FIG. 8. Higher magnification of a portion of the mucosal fold "a" of the preceding microphotograph. The central location of the large dilated lymphatic in this fold with the blood vessels situated between it and the surface of the mucosa is evident. However, note that a portion of the mucosal lymph plexus "a" and "b" is situated between these blood vessels and the surface of the fold and is separated from the epithelium by only a very thin layer of tissue. The close proximity of portions of the lymph plexus of the ampullar and fimbrial mucosa to the surface of the mucosa is of great importance in the present study. Carcinoma growing in the lymphatics of the mucosa may easily push through this thin layer of tissue and overlying epithelium into the lumen of the tube or into the peritoneal cavity, as will be shown. Carcinoma replacing the overlying epithelium in such an area may easily invade the nearby lymphatics. One of these superficial lymph vessels might take part in the formation of newly formed lymph vessels in granulation tissue arising on the surface of such an area of the mucosa (see Figs. 95 and 96). $\times 130$.

FIG. 9. Longitudinal section of a portion of the fimbriae of a normal appearing tube at a mucoserosal junction ("a"). The patient, A. H. No. 1698-32, parous and aged 45, had had the entire uterus and left tube and ovary removed and the pelvic floor repaired for descensus of the uterus associated with a follicular cyst of the ovary and a relaxed pelvic floor. The veins partially or completely filled with blood are evident. All of the empty spaces are lymphatics except "b," which is a small mesothelial cyst. The dilated lymphatics of the mucosa apparently drain into deeper lymph vessels of the tubal wall. Lymph vessels are not evident in the serosa at the right. The richness of the mucosal lymphatics and their fitness for the spread of carcinoma in them in this situation are evident. Their proximity to the surface of the mucosa exposes them to any pathological changes affecting the surface of the fimbriae. $\times 25$.



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PLATE 81

FIG. 10. Cross section of the ampulla of a tube from a patient, A. H. No. 4727-31, with carcinoma of the tubal mucosa and of the ovary secondary to that of the uterus as a result of lymphatic permeation from the uterine tumor (see also Figs. 18 and 19, and Figs. 5, 6 and 7 of two previous papers, ^{1,11}). Lymph vessels "a" of the mesosalpinx are filled with carcinoma. A collecting lymph vessel extending from the tube into the mesosalpinx, indicated by "b," demonstrates a channel by which the carcinoma in the lymph vessels of the mesosalpinx may have reached the mucosal lymphatics. The mucosal lymphatics are greatly distended with the carcinoma which in one situation ("c") has grown through the wall of the mucosal lymph vessel into the lumen of the tube. Note the scarcity of the subserosal lymphatics as compared with those of the mucosa if their injection with carcinoma is complete. Since carcinoma of the tubal mucosa and of the ovary may arise from carcinoma of the uterus by extension through the lymph vessels we would expect that carcinoma of the tubal mucosa might arise from the ovary through similar channels. $\times 16$.

FIG. 11. A portion of a cross section of the ampulla of the left tube in which the mucosal lymphatics are filled, as with an injection mass, with carcinoma secondary to that of the left ovary (Case 1). The distribution of the carcinoma in the mucosa is similar to that of the carcinoma in the preceding microphotograph. The pattern of the carcinomas in the mucosa is the same as that of the lymph vessels shown in Figures 2 and 3. Since the lymph vessels of the ampullar mucosa, shown in the preceding microphotograph, were injected with carcinoma by lymphatic permeation from a primary growth in the uterus it is conceivable that the carcinoma in the present section may have reached its present situation by a similar route from the ovary. $\times 54$.



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PLATE 82

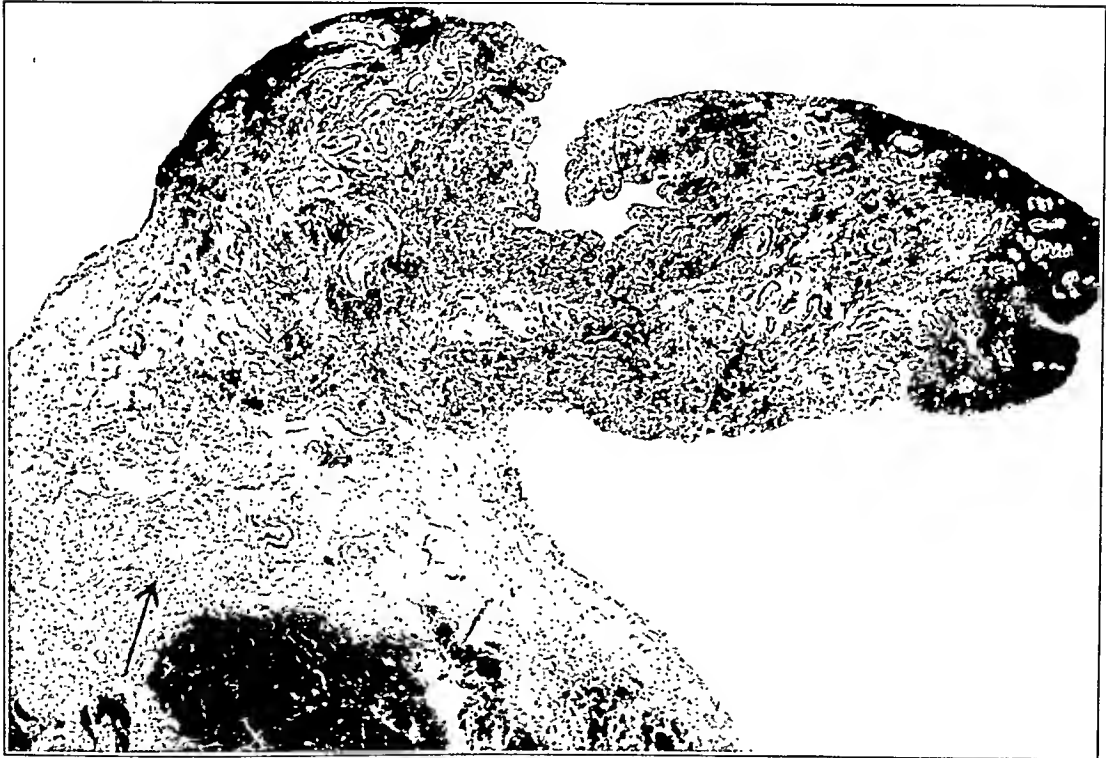
FIG. 12. Cross section of the entire tube, a portion of which is shown in the preceding microphotograph (lower magnification). Carcinoma is present in lymph vessels in all layers of the tube including the mesosalpinx "a," as in the tube shown in Figure 10. In places the growth has escaped from the lymph vessels and infiltrated the tubal tissues like the extravasation of an injection mass through a ruptured lymphatic. $\times 16$.

FIG. 13. Section of a small portion of the ovarian carcinoma of Case 1, the mesosalpinx and ovarian fimbriae. The fimbriae (above) are greatly thickened and distorted by carcinoma which has infiltrated their tissues. Carcinoma is also present in lymph vessels which accompany the blood vessels. The latter are very distinct, even with this low magnification. The ovarian carcinoma has invaded the tissues of the mesosalpinx and is barely visible in small lymph vessels (see arrow), apparently making its way toward the fimbriae above. $\times 10$.

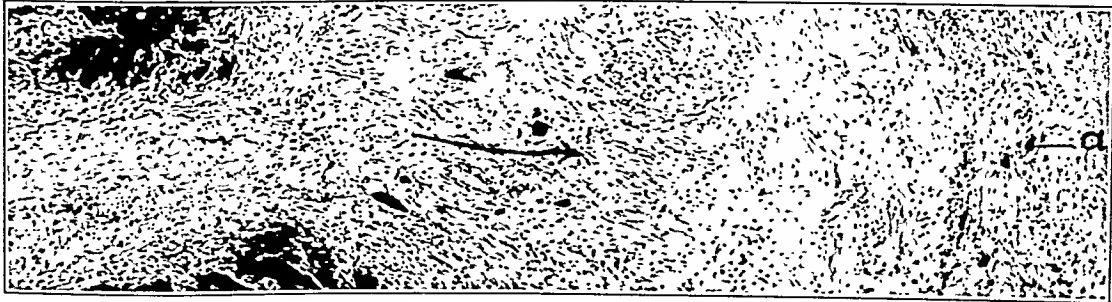
FIG. 14. Higher magnification of the field indicated by the arrow in the preceding microphotograph. (This print is mounted at right angles to the preceding one.) Above and below the arrow, carcinoma is shown in lymph vessels apparently extending from the ovarian carcinoma into the mesosalpinx. Carcinoma also is present in a lymph vessel ("a"). The condition found in the last two microphotographs indicates that the carcinomas in the lymphatics of the tubal mucosa shown in Figures 11 and 12 could have reached their present situation, through the lymph vessels, from the ovarian carcinoma. $\times 54$.



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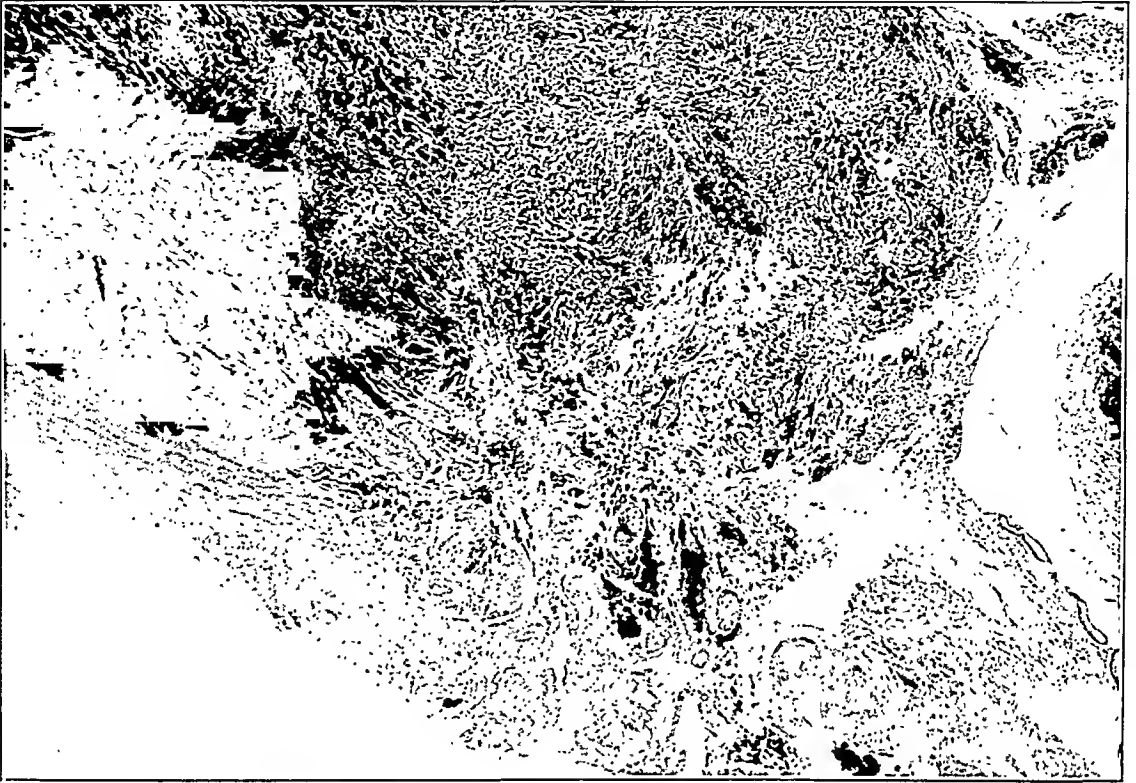


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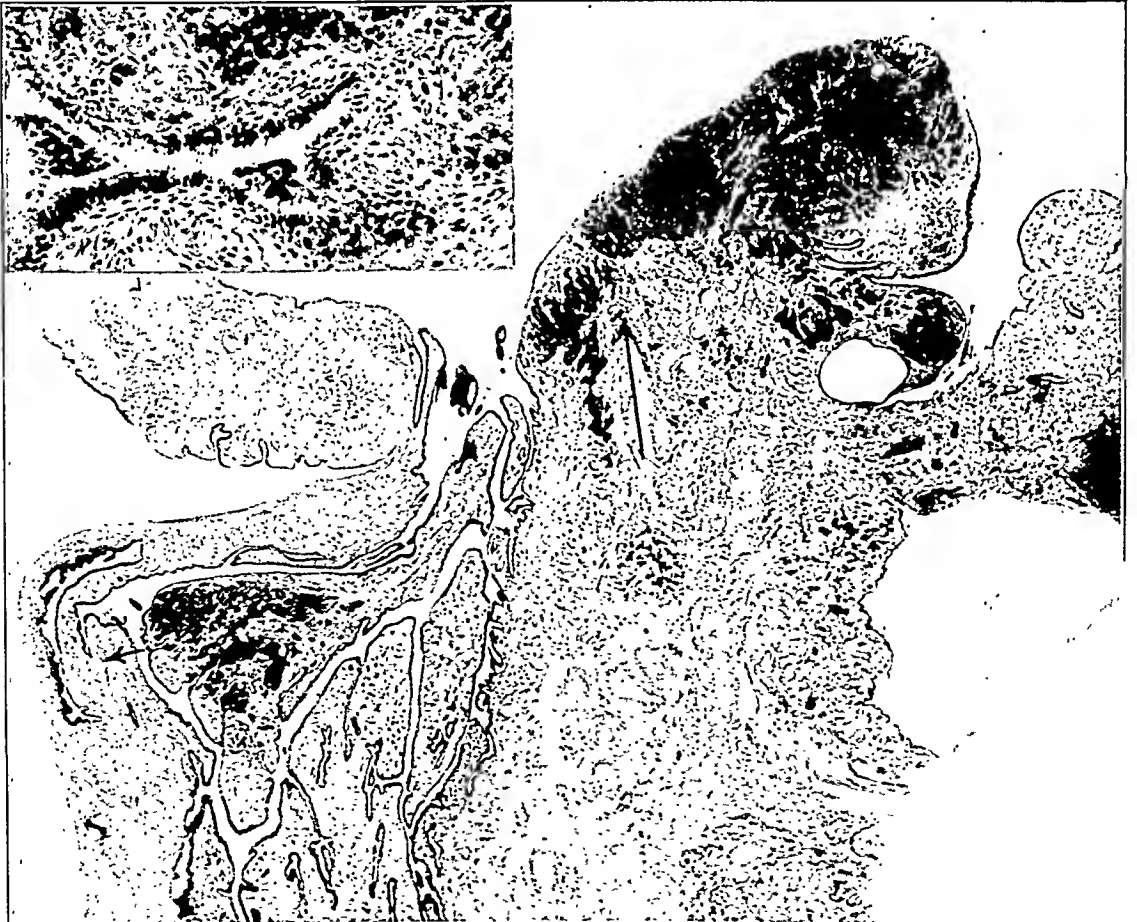
PLATE 83

FIG. 15. Section of a portion of the left ovary including its hilum (Case 2). It shows well the extension of the carcinoma beyond the ovary into the tissues of its hilum in lymph vessels which accompany the blood vessels. If secondary carcinoma is present in the mucosa of the accompanying tube, as the result of lymphatic permeation or metastasis, we would expect to find it primarily in the lymph vessels. $\times 10$.

FIG. 16. Longitudinal section of the distal portion of the left tube including its fimbriae (Case 2). The tissues of the fimbriae at the right and toward the ovary are infiltrated with carcinoma as in Figure 13. Carcinoma is present in many of the lymph vessels of the wall of the tube. The carcinoma to the left of the large arrow may well mark a lymphatic which normally drains the mucosal lymph vessels, and in this instance indicates a channel through which the carcinoma of the ovary may have reached the mucosa of the fimbriae. A mucosal fold of the ampulla at the left is also infiltrated with carcinoma ($\times 10$). The small arrow and the higher magnification ($\times 130$) of this field in the insert above demonstrate what I believe to be the outgrowth and extension of the carcinoma of the deeper tissues of the mucosa into the lumen of the tube and not the implantation of cancer cells on the surface of the mucosa and their subsequent invasion of the tissues of the mucosa.



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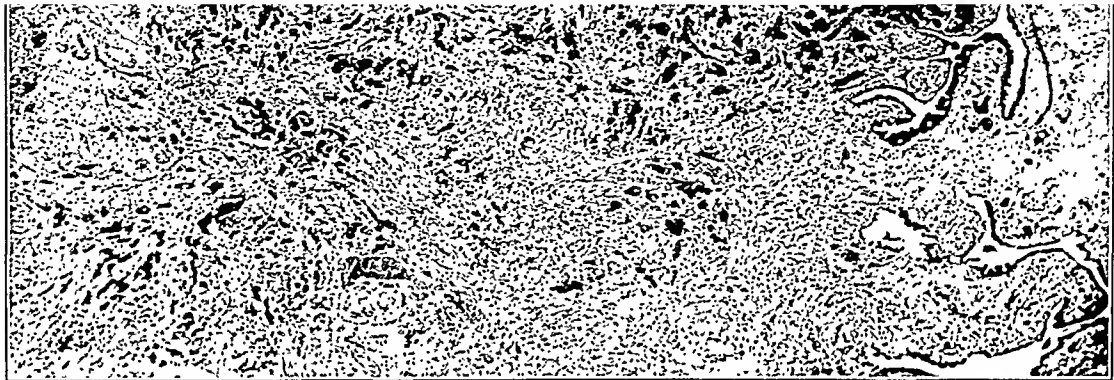
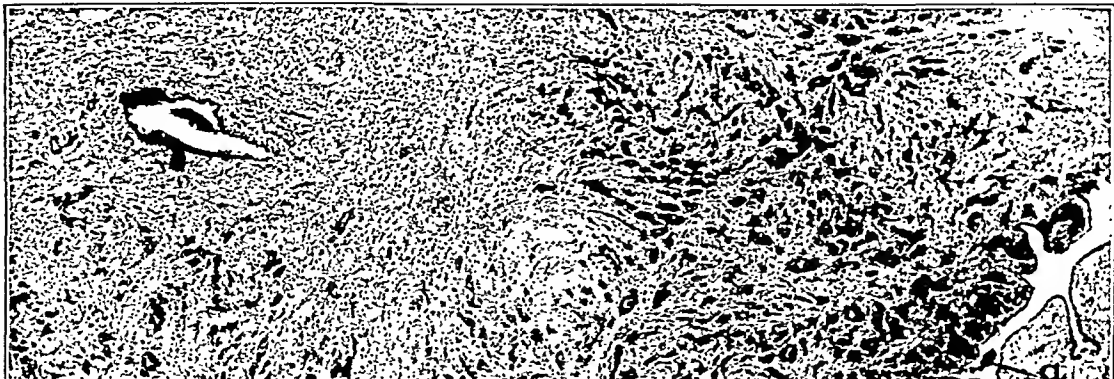


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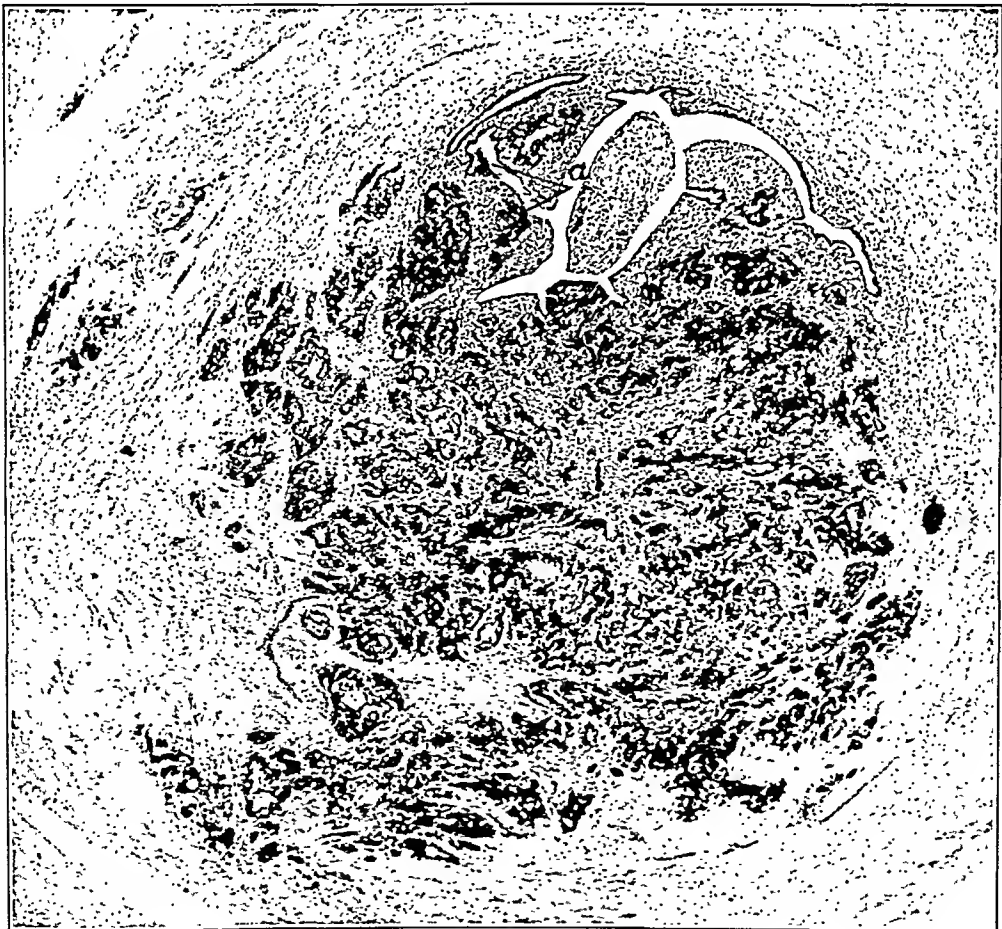
PLATE 84

FIG. 17. Longitudinal section of a portion of the proximal end of the ampulla of the tube shown in the preceding microphotograph. The wall of the tube is infiltrated by carcinoma which I believe is situated, for the most part, in lymphatics. The mucosa, at the right, is likewise infiltrated with carcinoma. Carcinoma "a" is present in the lumen of the tube and probably reached its present situation by the extension of the tumor in a mucosal lymph vessel through the overlying epithelium and is not a clump of cancer cells which escaped into the peritoneal cavity from the ovarian tumor and was subsequently swept into the lumen of the tube. All of the evidence found in the study of the material from this case points to the origin of the carcinoma of both the fimbrial and the ampullar mucosa by lymphatic permeation and metastasis from the ovarian tumor and not from the implantation of cancer cells on the tubal mucosa. Since ovarian carcinoma can reach the tubal mucosa through the lymph vessels, tubal carcinoma, both primary and secondary, might be able to reach the ovary by the same route. $\times 25$.

FIG. 18. Carcinoma of the proximal portion of the ampulla of the left tube secondary to carcinoma of the ovary (see the next microphotograph (Case 3)). The carcinoma in places ("a") has not only extended to the epithelium of the mucosa but has replaced it, as in Figure 16 (compare with the preceding microphotograph). I believe that the pathogenesis of the carcinoma of the ampullar mucosa in the two sections is the same. $\times 25$.



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Sampson

Implantation Carcinoma of Tubal Mucosa

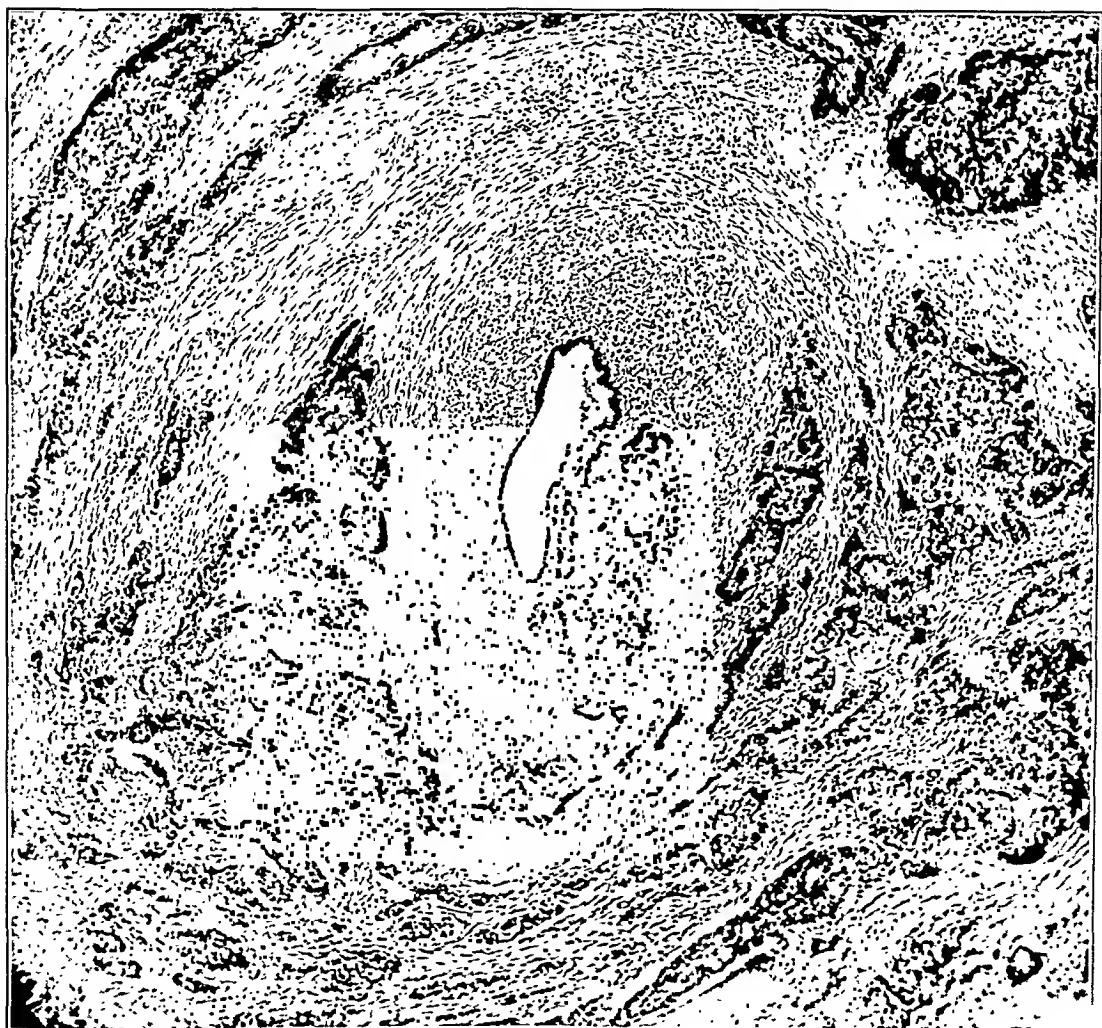
PLATE 85

FIG. 19. Coronal section of the left uterine cornu and the proximal portion of the tube including a portion of the ovarian carcinoma which has invaded both the uterus and the tube (Case 3). The ovarian carcinoma is partly necrotic and here it has failed to take the stain. The invasion of the uterine cornu, the mesosalpinx and walls of the isthmus of the tube by the growth is well shown. Carcinoma ("c") is also present in a lymph vessel of the mesosalpinx. Portions of the isthmus of the tube ("a and b") are necrotic and in both situations are surrounded by tissue infiltrated by the growth. The section shown in the preceding microphotograph of this case is from the same series of sections as this one. $\times 5$.

FIG. 20. Carcinoma of the mucosa of the uterine portion of the tube from the same series of sections as the one shown in Figure 18. The pathogenesis of the mucosal carcinoma in the two situations is the same. $\times 54$.



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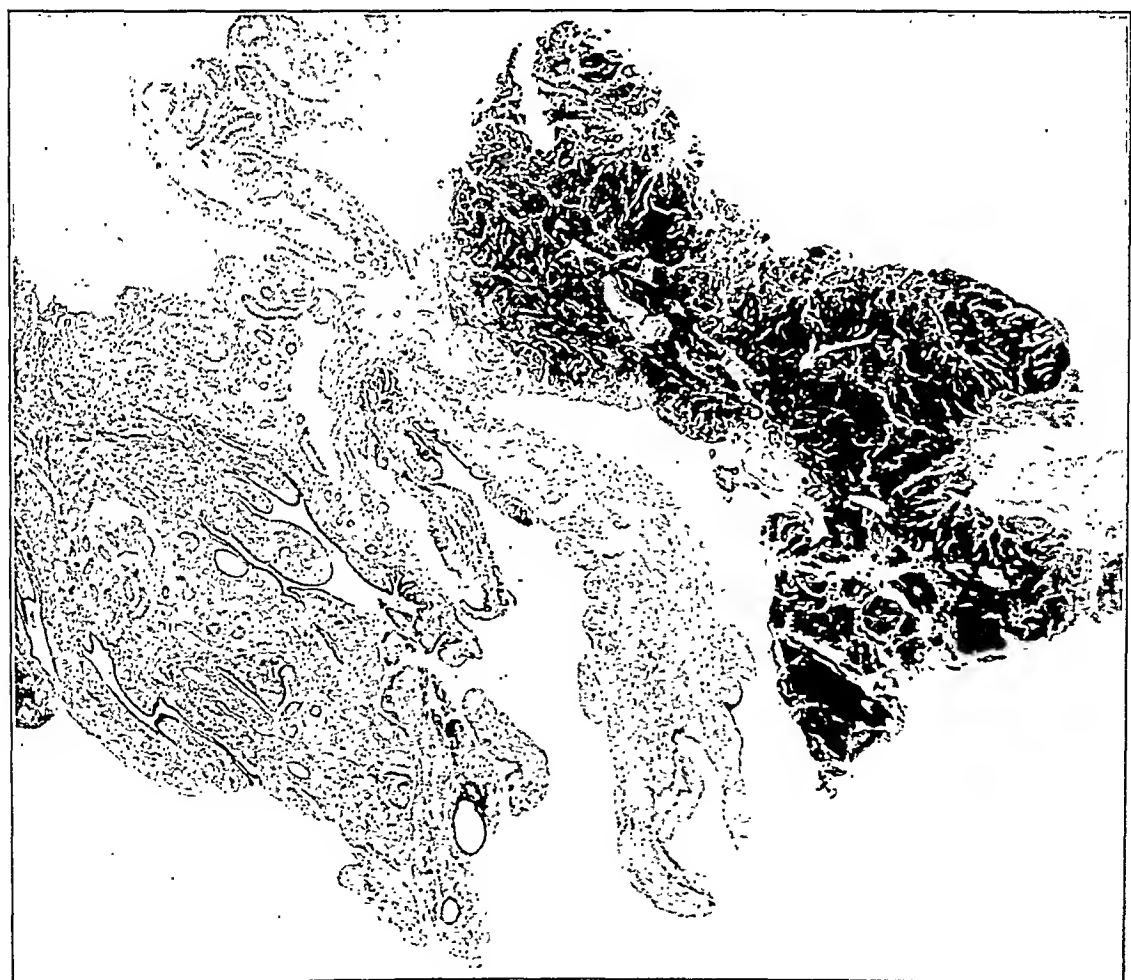
PLATE 86

FIG. 21. Cross section of the left tube and ovary from a patient with carcinoma of the left ovary associated with peritoneal carcinomatosis (Case 4). Carcinoma is present in the ovarian fimbriae adherent to the ovary and grossly appears to have arisen by direct extension from the growth in the ovary. Natural size.

FIG. 22. Longitudinal section of the tubal fimbriae shown in the preceding illustration through the carcinoma involving the ovarian fimbriae. The histological structure of the neoplasm in this situation is that of an advanced growth which has invaded the fimbriae from within and spread in their tissues as from lymphatic permeation (compare with Figs. 13 and 16). Carcinoma is present in lymph vessels in the tissues adjacent to the left hand portion of the tumor. An occasional small embolus of cancer cells can be found in lymph vessels in all parts of the tubal wall proximal to the fimbriae. The continuity of the fimbrial carcinoma with the primary ovarian growth, so clearly portrayed in the previous illustration that it must have been present, was not seen in the incomplete microscopic study of this area. $\times 10$.



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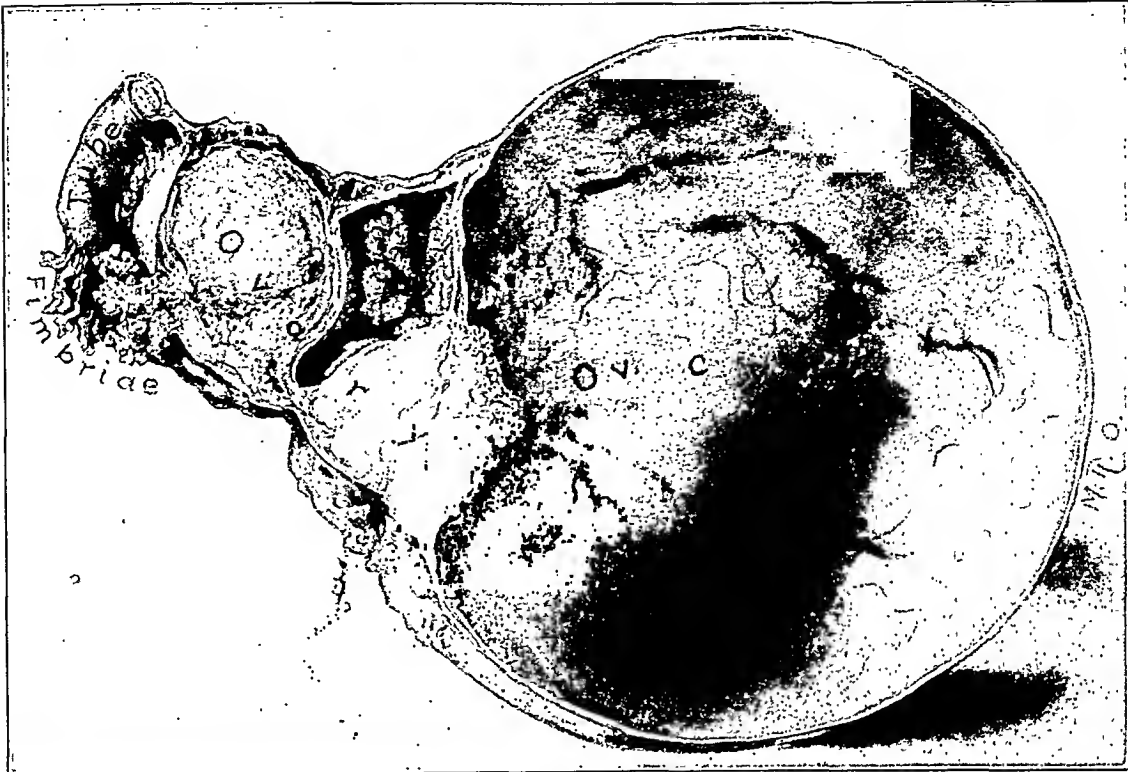


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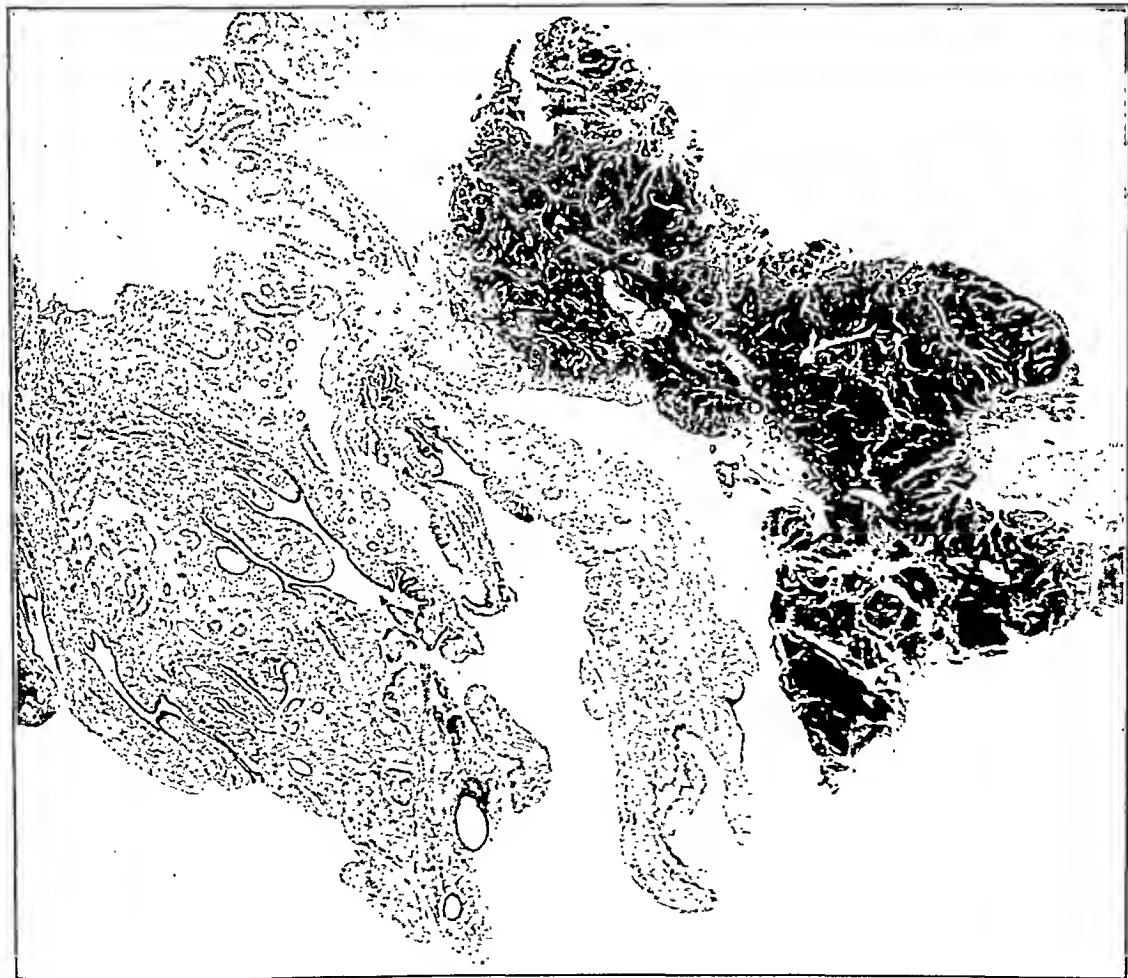
PLATE 86

FIG. 21. Cross section of the left tube and ovary from a patient with carcinoma of the left ovary associated with peritoneal carcinomatosis (Case 4). Carcinoma is present in the ovarian fimbriae adherent to the ovary and grossly appears to have arisen by direct extension from the growth in the ovary. Natural size.

FIG. 22. Longitudinal section of the tubal fimbriae shown in the preceding illustration through the carcinoma involving the ovarian fimbriae. The histological structure of the neoplasm in this situation is that of an advanced growth which has invaded the fimbriae from within and spread in their tissues as from lymphatic permeation (compare with Figs. 13 and 16). Carcinoma is present in lymph vessels in the tissues adjacent to the left hand portion of the tumor. An occasional small embolus of cancer cells can be found in lymph vessels in all parts of the tubal wall proximal to the fimbriae. The continuity of the fimbrial carcinoma with the primary ovarian growth, so clearly portrayed in the previous illustration that it must have been present, was not seen in the incomplete microscopic study of this area. $\times 10$.



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PLATE 87

FIG. 23. Coronal section through a tubo-ovarian cyst showing multiple carcinomas of various sizes of the lining of the ovarian cyst, and also multiple, very small carcinomas of the mucosa of the ampulla of the tube (Case 5). The multiple tumors in both the ovary and the tube indicate that they are of either multicentric origin or one parent growth with the other tumors secondary. The great variation in the size of the tumors, most evident in the ovarian cyst, suggests a variation in their ages. This supports the theory of a primary growth with metastases, but does not exclude the possibility of the same carcinogenetic factors activating the epithelial lining of both the ovarian cyst and the tube at different times. The epithelial lining of the ovarian cyst is continuous with and histologically indistinguishable from the epithelium of the tube. The tumors are all superficial. Carcinoma was not found in the lymph vessels of either the ovary or the tube. If a primary growth is present it obviously should be the largest one in the ovary. Natural size.

FIG. 24. One of the small discrete tumors of the ovarian cyst. It is a typical papillary carcinoma which, in this situation, has spread over the lining of the cyst by replacing the epithelium of the latter. Tumor cells may readily escape from such a growth into the cavity of the ovarian cyst as well as from the largest and judged parent tumor shown in the preceding illustration. Similar cells would also migrate into the lumen of the patent tube (see arrow in Figure 23). $\times 5$.

FIG. 25. Section of the tubo-ovarian cyst at the tubo-ovarian junction, corresponding to the field bounded by the dotted lines and the cavity of the tubo-ovarian cyst, marked "a" in Figure 23. Multiple small papillary tumors of various sizes are shown on the lining of the ovarian cyst at the left which in this situation consists, at least in part, of tubal fimbriae fused with the wall of the cyst (see pointer "a"). Similar papillary tumors are present also on the tubal mucosa, above and at the right. $\times 8$.

FIG. 26. A portion of the ampulla of the tube; from a block adjacent to the preceding one, demonstrating the further spread of the tumors on the mucosa of the tube. $\times 10$.



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FIG. 27. Granulation tissue which has arisen on the surface of the tubo-ovarian cyst through breaks in its epithelial lining. This tissue arose from the portion of the ovarian cyst lined by tubal fimbriae. The distal portion of this outgrowth is shown, in the series of sections, to be attached to the tip of a judged mucosal fold of the fimbriae. Note the sessile round polypoid mass of newly formed tissue marked "a" and the pedunculated mass of younger granulation tissue above it. Outgrowths of tissues, like these, are frequently seen on the serosa of patients with peritoneal carcinomatosis. Cancer cells floating about in the peritoneal cavity become attached to and embedded in these outgrowths. Since this tissue forms the stroma of one type of peritoneal implant it might also form the stroma of a similar type of mucosal implant. $\times 54$.

FIG. 28. The distal and youngest portion of the newly formed tissue shown in the preceding microphotograph from another section in this series. A clump of cancer cells is embedded in this tissue which consists of fibrin and cells derived from the subepithelial tissues of the fimbrial mucosa. The majority of these migratory cells are judged to be fibroblasts. A few polymorphonuclear leukocytes and lymphocytes are present also. Newly formed blood vessels have not yet appeared in the tissue shown here. Phenomena like this are frequently encountered in patients with peritoneal carcinomatosis and present early stages in the pathogenesis of one type of carcinomatous implant. $\times 130$.

FIG. 29. A pedunculated polypoid carcinomatous implant on the mucosa of the ampulla of the tube, shown in Figure 23, near the isthmus. Clumps of cancer cells are embedded in the body of this implant. Its slender vascular pedicles are similar to those of the polypoid outgrowth of granulation tissue shown in Figure 27. The pathogenesis of this implant is suggested in the two preceding microphotographs. Polypoid implants, like this one, are frequently encountered on the serosa of patients with peritoneal carcinomatosis where all stages in their development have been studied.⁹ $\times 54$.

FIG. 30. The body of a pedunculated polypoid outgrowth of newly formed tissue (see "a" of Fig. 26), which has arisen from the mucosa of the ampulla of the tube. The histological structure of this tissue is similar to but older than the sessile, newly formed tissue marked "a" in Figure 27. Clumps of cancer cells are splinted by fibrin on the surface of this tissue, at the right. The condition shown here represents an early stage in the possible implantation of cancer cells on newly formed tissue derived from the tubal mucosa (see the next microphotograph). The pedicle which unites this tissue to the tubal mucosa beneath it doesn't appear in this section. The black area marked "a" may be the remains of cancer cells which became embedded in this tissue but failed to survive (see Fig. 39). $\times 130$.

FIG. 31. The body of a pedunculated polypoid outgrowth of newly formed tissue similar to the tissue shown in the preceding microphotograph and, like the latter, arising from the mucosa of the ampulla of the tube. Carcinoma is growing on the surface of this tissue which is apparently younger than the tissue shown in Figure 30. I believe that the condition shown here represents a stage in the development of one type of polypoid implant later than that illustrated in the preceding microphotograph. The slender vascular pedicle of this implant appears in Figure 40. $\times 130$.



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FIG. 32. Carcinoma ("a") growing on the surface of the tip of a mucosal fold of the fimbriae in the field marked "a" shown in Figure 25 but from another section in this series. Note that the epithelium covering this portion of the fold has almost entirely disappeared, thus creating a soil suitable for the implantation and growth of cancer cells. Even some of the fibrin ("b" and "c") which may at one time have held the cancer cells against the tubal mucosa, remains (see Fig. 30). The condition shown here represents an early stage in the growth of cancer cells implanted on the tubal mucosa without the assistance of polypoid granulation tissue as observed in Figures 30 and 31. $\times 130$.

FIG. 33. A stage in the growth of carcinoma implanted on the tip of a mucosal fold of the tube later than that shown in the preceding microphotograph, from the same series of sections as the section shown in Figure 26. Fibrin ("a") which at one time may have splinted the cancer cells on the tubal mucosa still remains. $\times 130$.

FIG. 34. A still later stage in the growth of carcinoma implanted on the tip of a mucosal fold of the ampulla, from the same series of sections as the preceding one. The papillary structure of this growth is more evident than the one shown in Figure 33. However, the implant is still immature (see the mature implants shown in Figs. 35 and 41). Note a sharp demarcation between the carcinoma and the tubal epithelium and that the carcinoma does not present the appearance of having arisen from that epithelium. $\times 130$.

FIG. 35. Higher magnification of the ampullar mucosa with carcinoma on its surface marked by pointer "b" in Figure 26. The carcinoma, at the left, is splinted against the mucosa by fibrin. For a higher magnification of this field see the next microphotograph. One may judge that the reaction of the subepithelial tissue of the mucosa is caused by the carcinoma implanted already on the tip of a slender mucosal fold, similar to the carcinoma in Figure 32. Later it might have become implanted on the mucosa, shown here, if the patient had not been operated upon. The carcinoma at the center and right represents a mature implantation of cancer cells on the tubal mucosa. $\times 105$.

FIG. 36. Higher magnification of the left hand portion of the field shown in the preceding microphotograph. The fibrin, splinting the carcinoma, has been invaded by cells derived from the subepithelial tissues of the mucosa beneath the carcinoma. The majority of these migrating cells are judged to be fibroblasts. A few polymorphonuclear leukocytes and lymphocytes are present. These cells are passing through and between the epithelial cells which may be injured by the nearby carcinoma, thus permitting the escape of fibrin and migratory cells into the lumen of the tube. $\times 170$.

FIG. 37. Higher magnification of the central portion of the tubal mucosa shown in the next microphotograph. Clumps of embolic cancer cells are splinted on this mucosa by fibrin. At the left, the tubal epithelium has disappeared. A clump of cancer cells ("a") is in contact with the resulting raw area, but may not be actually implanted in this situation. However, to the right of the center another break in the tubal epithelium occurs (see pointer "b") with carcinoma definitely implanted on this area of the mucosa. A later stage in the phenomena shown here may result in the condition pictured above in Figure 35. $\times 130$.



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PLATE 90

FIG. 38. Carcinoma of the tubal mucosa from the same section shown in the preceding microphotograph. Note the tall polypoid growth with a long slender pedicle. This pedicle is covered with tubal epithelium. I believe that this tumor is a mature implant either on the tip of a slender mucosal fold or on polypoid, pedunculated, newly formed tissue (see Fig. 40). If on the latter, the tubal epithelium has grown over the surface of the pedicle of the implant. The judged early implantation of embolic clumps of cancer cells, shown in Figure 37, appears in the center of this field. A portion of a mature implant, similar to the one shown in Figure 35, appears at the right. $\times 54$.

FIG. 39. Cancer cells, singly and in clumps, enmeshed in fibrin with evidence of the death of two of the cancer cells in the largest clump. The cancer cells in the clump marked "a" also appear to be dead. Compare with the judged living cancer cells below them. In the majority of instances in this specimen the cancer cells on the surface of or enmeshed in fibrin stained as well as the tubal epithelium and were judged to be alive when the patient was operated upon. Apparently they may live a long time in the lumen of the tube without implantation on vascular tissue. Evidence of mitosis was not observed in any of these cells nor was it detected in the many carcinomas of the tubal mucosa which were carefully examined for this phenomenon. $\times 170$.

FIG. 40. The early implantation of cancer cells on polypoid granulation tissue arising from the tubal mucosa. This is the same early implant shown in Figure 31, from another section in this series. The greater portion of its long, slender vascular pedicle appears in this section (compare also with Figures 27 and 29). Had the epithelium of the tube grown over the pedicle of this implant it later might have presented a picture similar to that shown above in Figure 38. Clumps of judged dead cancer cells are marked "a." $\times 107$.

FIG. 41. Higher magnification of the mature implant marked "b" in Figure 25. It evidently arose from the growth of cancer cells implanted either on the tip of a mucosal fold, as shown in Figures 32, 33 and 34, or on the surface of polypoid, newly formed tissue, similar to that pictured in the preceding microphotograph. Clumps of viable cancer cells might easily escape into the lumen of the tube from the tips of the growing papillary processes of this and other similar neoplasms shown in this tubo-ovarian cyst. $\times 107$.



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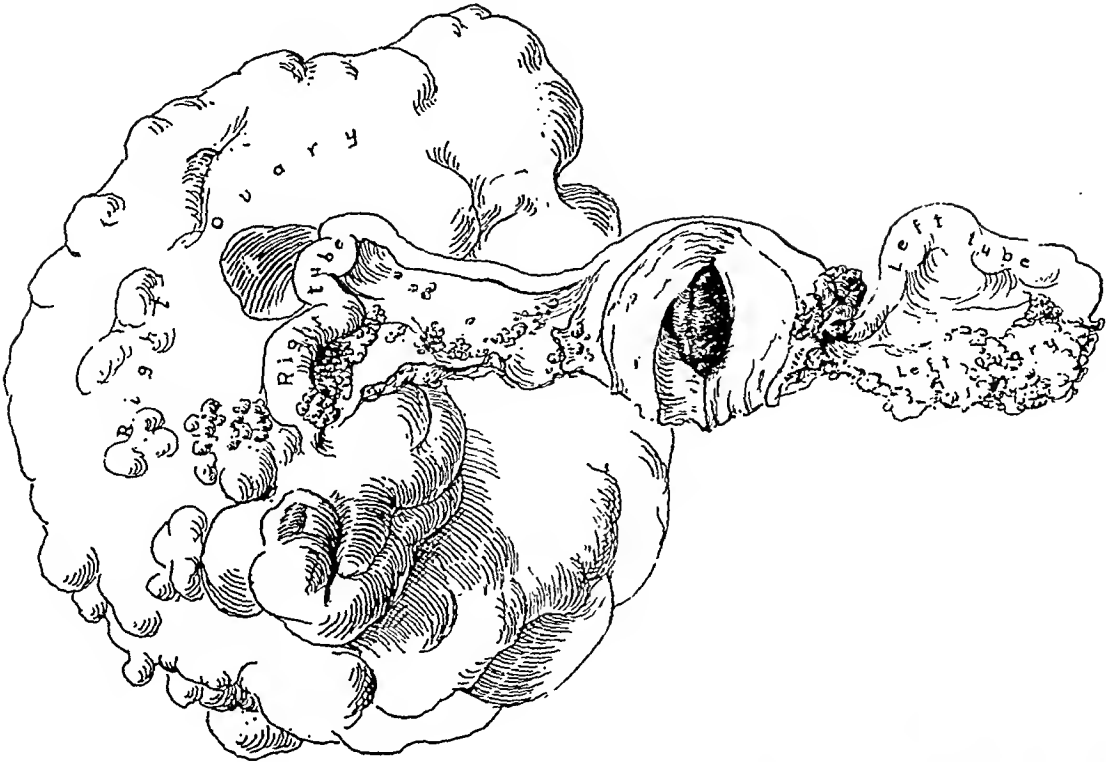
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PLATE 91

FIG. 42. Anterior view of the body of the uterus and both tubes and ovaries from a patient (Case 6) with carcinoma of both ovaries and a peritoneal carcinomatosis. The right ovary is replaced by a large carcinoma. The right tube is adherent to the ovarian tumor with the fimbriated end embedded in it. The left ovarian tumor was much smaller than the right one, but was so adherent to the side of the pelvis that only a portion of it was removed. The fimbriae of the left tube are partially embedded in the ovarian tumor of that side. Small carcinomatous implants can be seen on the anterior surface of the right broad ligament and a large one on the anterior surface of the left uterine cornu. A large benign mucosal polyp is present in the uterus. $\times 3/5$.

FIG. 43. A portion of the left ovarian tumor, shown in the preceding illustration, which has invaded the fimbriae of the left tube. At the left the carcinoma is advancing, like a primary growth in this situation, by replacing the epithelium of the mucosal folds. At the right the carcinoma has already partially or completely destroyed some of the mucosal folds. $\times 25$.

FIG. 44. The attempted implantation of cancer cells on a mucosal fold of the fimbriae of the left tube. A clump of cancer cells "a" is resting on the surface of a fold. While the mucosal epithelium beneath the tumor cells is lacking there is no appreciable evidence of any reaction of the tissues of the fold in this situation. Cancer cells "b" are shown attached to a strand of granulation tissue which has arisen on the surface of the fold. The reaction, present here, suggests that this attempted implantation might have taken place. $\times 130$.



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PLATE 92

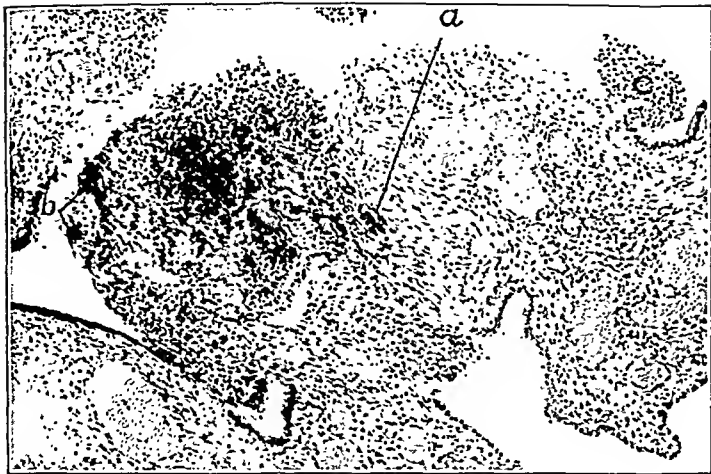
FIG. 45. A mucosal fold of the fimbriae of the left tube (Case 6) with a large sessile polypoid growth of granulation tissue on its surface, at the left. This tissue is identical with the tissue frequently found on the surface of the serosa of patients with peritoneal carcinomatosis where it occurs without and also with cancer cells attached to its surface or embedded in it. This tissue forms the stroma of many peritoneal implants and plays an important rôle in their pathogenesis. In the present situation it has developed through breaks in the epithelial covering of the mucosal fold. A small clump of epithelial cells ("a") is included in the base of this tissue. Recognizable cancer cells were not detected in this tissue. However, possible dead cancer cells are present on its surface at "b." At the right a small polypoid growth of newly formed tissue ("c") is present on the surface of the fold. $\times 54$.

FIG. 46. Polypoid granulation tissue on the surface of a mucosal fold, which has developed through a break in the epithelial covering of the fimbriae of the same tube shown in the preceding microphotographs. Cancer cells ("a") are embedded in this tissue. This lesion demonstrates an early stage in one type of implantation of cancer cells on the epithelial covered fimbrial mucosa identical with that often encountered on the surface of the mesothelial covered serosa of patients with peritoneal carcinomatosis (see Figs. 99 and 101). $\times 54$.

FIG. 47. Granulation tissue on the surface of a mucosal fold of the fimbriae from the same series of sections as those shown in the preceding microphotographs. This newly formed tissue has surrounded the carcinoma implanted on the surface of the fimbrial mucosa. At the right of this implant and fused with it more recent granulation tissue has developed. A clump ("a") of viable appearing cancer cells and two clumps ("b") of possible dead cancer cells are embedded in this latter tissue. The last three microphotographs demonstrate that cancer cells escaping from an ovarian carcinoma into the peritoneal cavity may become embedded in granulation tissue arising on the epithelial covered fimbrial mucosa just exactly as they become embedded in similar tissue arising on the mesothelial covered peritoneum. The etiology of this granulation tissue in the two situations should be the same. $\times 54$.

FIG. 48. A mature polypoid carcinomatous implant on the surface of an epiploic appendage, from the same patient as the preceding ones. It presents a stage in the implantation of cancer cells later than the one shown in the preceding microphotograph. $\times 25$.

FIG. 49. A mature, pedunculated, polypoid carcinomatous implant on the serosa of the right tube, also from the same patient. The pathogenesis of these two serosal implants undoubtedly is the same as the pathogenesis of the mucosal implants just demonstrated. $\times 25$.



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Sampson

Implantation Carcinoma of Tubal Mucosa

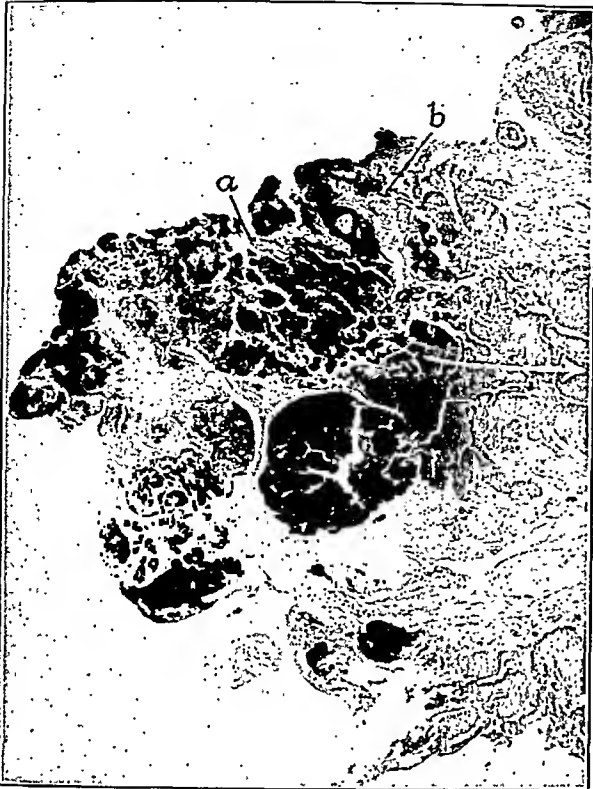
PLATE 93

FIG. 50. Longitudinal section of the distal end of the right tube shown in Figure 42. This end of the tube was embedded in the carcinoma of the right ovary. The ovarian growth has invaded the fimbriated end of the tube from all sides and has replaced nearly all of the mucosal folds of the fimbriae. The intact tips of two mucosal folds appear at "a" and "b." By continuous extension through the abdominal ostium of the tube (see arrow) the carcinoma has invaded and distended the lumen of the ampulla of the tube (see the next microphotograph). $\times 5$.

FIG. 51. A cross section of the ampulla of the tube shown in the preceding section and near the latter. The invasion of the outer portion of the wall of the tube by the ovarian carcinoma is shown at the left. The lumen of this portion of the tube is distended by the tumor which has grown through the abdominal ostium (see the preceding microphotograph). Note that the tumor is penetrating the lumen of the tube by replacing its mucosa and has not invaded the wall of the tube in this situation. $\times 4$.

FIG. 52. An oblique section of the tube demonstrating the continuation of the tumor, shown in the preceding microphotograph, through the lumen of the tube toward the uterus (from a block following the one from which the preceding section was taken). Ovarian carcinoma appears at the bottom of the field where it has invaded the mesosalpinx. At the left the lumen of the tube is partially filled by carcinoma which is a continuation of the growth shown in the preceding section and which has advanced by replacing the tubal mucosa. At the right a still further extension of the growth is indicated. Carcinoma "b" is a continuation of carcinoma "a." Sections of tips of the advancing tumor appear as large emboli of cancer cells in the lumen of the tube. Small true emboli of cancer cells, however, are scattered throughout the lumen of the tube. At "c" isolated carcinoma appears (see the next microphotograph). $\times 5$.

FIG. 53. Higher magnification of the area marked by the pointer "c" in the preceding illustration (section reversed). Carcinoma is shown replacing the tubal mucosa at the right and embedded in granulation tissue at the left. What is the origin of the carcinoma in this situation? See the following five microphotographs, and compare with Figure 47. $\times 54$.



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PLATE 94

FIG. 54. Another isolated carcinoma of the tubal mucosa from the same series of sections as the ones shown in the two preceding microphotographs. Granulation tissue has developed through breaks in the epithelium of the tubal mucosa similar to that shown in Figure 45 and also similar to the granulation tissue frequently encountered on the serosa of patients with peritoneal carcinomatosis. Evidently cancer cells floating about in the lumen of the tube became attached to the surface of this granulation tissue and have grown here. $\times 54$.

FIG. 55. Isolated carcinoma on the tip of a secondary fold of the tubal mucosa from a portion of the tube nearer the uterus than the implant shown in the preceding microphotograph. The carcinoma in this situation must be derived either from the epithelium of the tubal mucosa (an instance of multicentric origin) or be metastatic from the growth which has invaded the lumen of the tube. The reaction of the tubal mucosa with a loss of its epithelium and a proliferation of the subepithelial tissue cells would furnish a fertile field for the implantation or grafting of cancer cells floating in the lumen of the tube. Compare this microphotograph with the preceding one and also with Figures 33 and 34. The histological picture of the lesions in the different situations is very similar. $\times 54$.

FIG. 56. The same implant pictured in the preceding microphotograph and near the latter in the series of sections. It shows the relation between the tumor cells and the epithelium of the mucosa better than the preceding section. $\times 54$.

FIG. 57. Another implant on a secondary fold of the tubal mucosa. This one is apparently younger than the preceding ones and from the same series of sections. There is an accumulation of lymphocytes in the tissues of the mucosal fold beneath the upper pole of the tumor. This is an evidence of a tissue reaction which may have arisen either before or after the tumor developed. Note that the tumor presents the histological picture of having been added to the mucosa rather than of having arisen from its epithelium. $\times 54$.

FIG. 58. Two implants similar to the preceding one and also from the same series of sections. The larger one is evidently an older implant. The smaller tumor ("a") apparently is a very early one. Although many secondary carcinomas of the tubal mucosa, similar to those shown in the last four microphotographs, were present in this tube, the initial stage of their pathogenesis, which is so well shown in the preceding case, was not detected. $\times 54$.



54



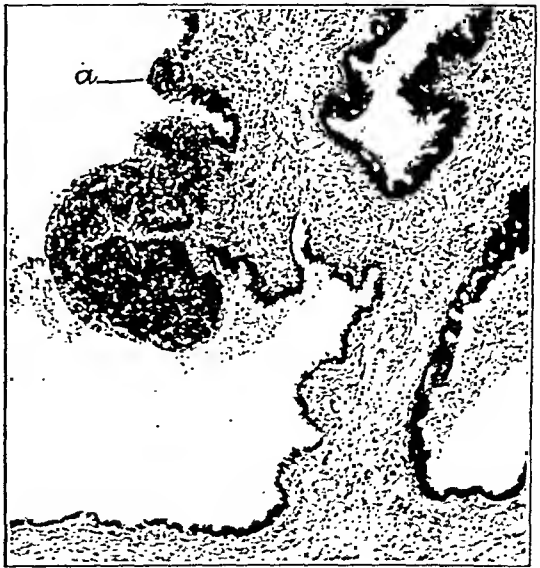
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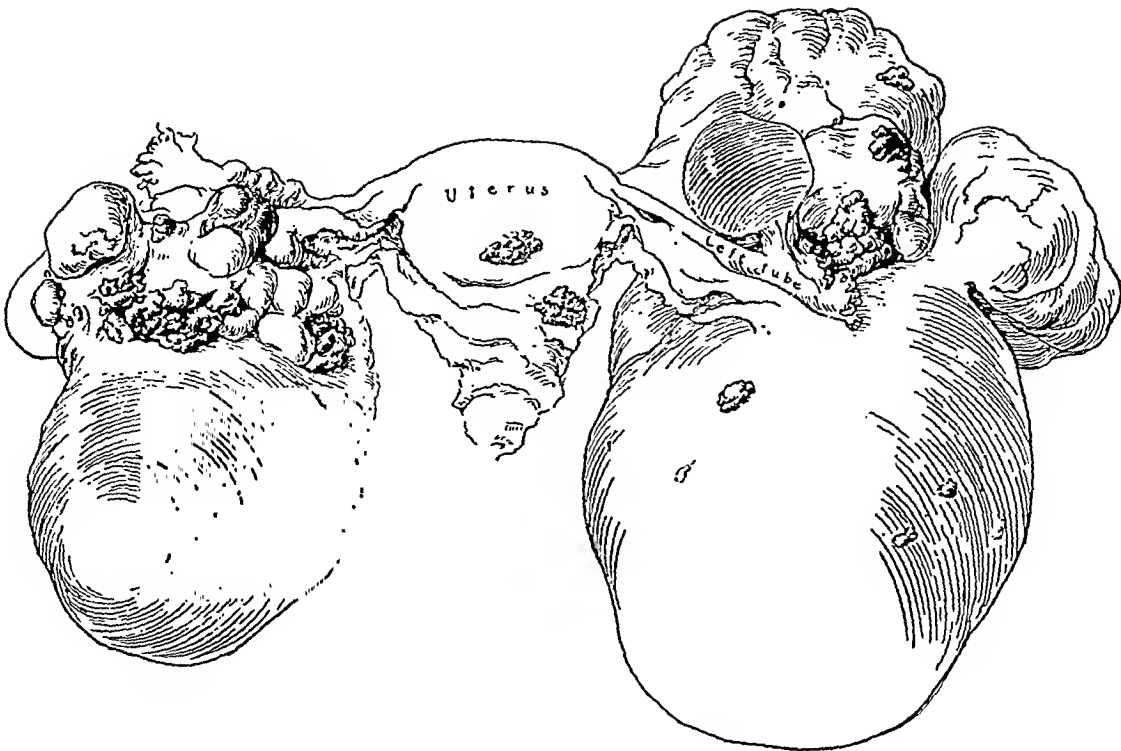
PLATE 95

FIG. 59. Anterior view of the uterus, tubes and ovaries from a patient with adenocarcinoma arising in a papillary adenocystoma of both ovaries associated with an extensive peritoneal carcinomatosis (Case 7 of present paper). All stages in the development of various types of polypoid peritoneal implants were encountered in the study of the peritoneal metastases in this case (see Case 4 of previous paper¹⁰). Newly formed lymphatics were found in the stroma of some of these implants. The gross appearance of this specimen, with implants on the anterior surfaces of the uterus, left broad ligament and ovaries, is shown in this drawing. The fimbriae of the tubes appear normal on casual examination (see Figs. 63 and 69). $\times 2/3$.

FIG. 60. Section of sediment obtained by centrifugalizing some of the ascitic fluid removed from the patient with the ovarian carcinomas shown in the preceding illustration. The clumps of deeply stained cancer cells are easily discernible. Cells similar to these might become implanted on the peritoneum and the fimbrial mucosa if conditions favorable for this phenomenon arose in these situations (see Figs. 61, 62 and 65). $\times 130$.

FIG. 61. The tip of a polypoid outgrowth of early granulation tissue which has arisen on the surface of the vesico-uterine reflection of peritoneum from Case 7. A portion of this peritoneum was excised and placed in formalin before proceeding with the major part of the operation. Clumps of cancer cells, similar to those shown in the preceding microphotograph, are becoming attached to and enmeshed in the tip of this tissue, its youngest and most actively growing portion. They are not found in any other portion of this outgrowth of granulation tissue. $\times 54$.

FIG. 62. Another outgrowth of granulation tissue similar in situation and form to the preceding one and possibly of the same age. It presents a stage in this type of cancer cell implantation a little later than the one just shown. This granulation tissue arose from the serosa in response to stimulation caused, in some way, by cancer cells which had escaped into the peritoneal cavity. The granulation tissue in both outgrowths is abundantly supplied with newly formed blood vessels. Yet, in spite of a careful study of many sections of these two outgrowths, lymph vessels cannot be recognized in them. However, they may be present. $\times 54$.



59



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62

FIG. 63. The fimbriae of the right tube shown in Figure 59. A careful examination of this tube under water, with a hand lens, demonstrated three small nodules, "a," "b" and "c," which are attached to the mucosal folds of the fimbriae and resemble very closely many of the polypoid implants on the peritoneum of this and other patients with peritoneal carcinomatosis of ovarian origin. These nodules might easily be missed in a casual inspection of the fimbriae (see Fig. 59). $\times 1\frac{1}{2}$.

FIG. 64. Longitudinal section of the fimbriated end of the tube shown in the preceding illustration. A small portion of a pedunculated polypoid implant is indicated by the letter "a," a sessile polypoid implant by "b" and a pedunculated one by "c." A portion of the slender pedicle of implant "c" can be seen pointing upward and to the right. These implants correspond to the three nodules shown in the preceding illustration. $\times 5$.

FIG. 65. A pedunculated polypoid outgrowth of granulation tissue, with clumps of cancer cells becoming implanted in it, is shown between two mucosal folds of the fimbriae. This implant is marked "a" in the preceding microphotograph but from another section in this series. This outgrowth of granulation tissue arose from the subepithelial tissues of a mucosal fold of the fimbriae just as the polypoid outgrowths of granulation tissue shown in Figures 61 and 62 arose from the submesothelial tissues of the serosa of the vesico-uterine fold of peritoneum in the same patient. The pathogenesis of the granulation tissue in the two situations is undoubtedly the same. Cancer cells, floating about in the ascitic fluid present in this patient, are becoming implanted in newly formed granulation tissue in both situations. Portions of the slender pedicle of this outgrowth of granulation tissue can be seen extending downward toward the mucosal fold below it (see Fig. 67). $\times 25$.

FIG. 66. Higher magnification of polypoid implant "b" shown in Figure 64 (from the same section) and also of the mucosal fold to which it is attached. This implant is sessile and much older than the one shown in the preceding microphotograph. $\times 25$.

FIG. 67. A cross section ("a") of the pedicle of the polypoid outgrowth of granulation tissue shown in Figure 65 (from another section in this series) and a portion of the underlying mucosal fold from which it arises. Blood vessels can be seen readily in the cross section of this pedicle. Lymph vessels can not be detected in it although they may be present. They are very evident in the adjacent fimbrial mucosa from which the granulation tissue arose. $\times 54$.

FIG. 68. A higher magnification of implant "c" seen in Figure 64 (from another section in this series) including a cross section ("a") of its long slender pedicle and the tip of the mucosal fold from which the pedicle arises, below it and to the right of the body of the implant. Blood vessels can be seen readily, especially under higher magnification, in this pedicle, but lymph vessels can not be detected in it. Cross sections of these pedicles are very characteristic and resemble cross sections of small umbilical cords. It is obvious that this implant represents a later stage of the process shown in Figure 65. $\times 54$.



63



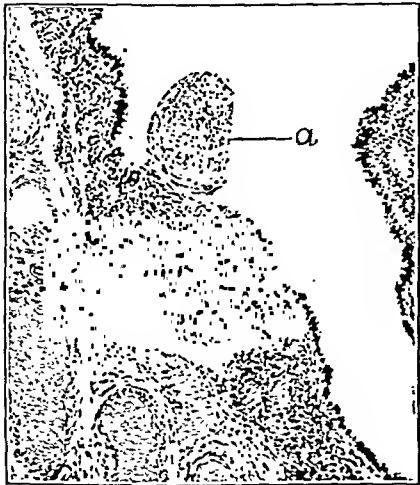
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66



67



68

Sampson

Implantation Carcinoma of Tubal Mucosa

PLATE 97

FIG. 69. The distal end of the left tube illustrated in Figure 59. A pedunculated polypoid implant ("a") is shown attached to the serosa of the ampulla of the tube. A similar implant ("b") is attached to the mucosa of the fimbriae. Implant "a" was injured in blocking the tube. Sections of a polypoid serosal implant similar to implant "a" and from the same patient are shown in Figures 60 to 62 inclusive of a previous paper.²⁰ $\times 1\frac{1}{2}$.

FIG. 70. Longitudinal section of the fimbriated end of the tube shown in the preceding illustration (compare with Fig. 64). The mucosal implant "b" of Figure 69 appears for its entire length, including its long slender vascular pedicle and the attachment of the latter to the mucosa of the fimbriae. $\times 5$.

FIG. 71. Higher magnification of the lower portion of the body of the implant and a portion of its pedicle shown in the preceding microphotograph. Blood vessels filled with blood may be followed, in this series of sections, from preexisting blood vessels of the fimbriae through the entire length of the pedicle up into and throughout the stroma of the implant proper. Lymph vessels, with interruptions, may be followed in like manner from those in the fimbrial mucosa through the pedicle and into the base of the implant proper. However, these cannot be detected in the dense tissues of the latter. Lymph vessels or branches of the same vessel are marked by the pointers "a," "b" and "c." A few lymphocytes are present in the lumens of vessels "b" and "c." Carcinoma is present between and to the right of the upper portion of these two vessels and later might have invaded these vessels and gained access to the lymph channels of the fimbriae to which the pedicle is attached if the patient had not been operated upon. $\times 54$.

FIG. 72. Higher magnification of a portion of the fimbrial mucosa at the attachment of the pedicle of the above described implant (see Fig. 70) but from another section in this series. Only a small portion of the pedicle appears in this section. A judged partially collapsed lymph vessel ("a") can be just detected in this portion of the pedicle. Blood vessels cut obliquely and longitudinally can be seen above the lymph vessel. There was no difficulty in following the blood vessels, easily seen in the series of sections, from preexisting blood vessels of the mucosa into and through the pedicle of the implant and into its body. However it is impossible to establish the continuity of the judged lymph vessels of the pedicle with the nearby, easily discernible lymph vessels of the fimbrial mucosa. This difficulty is frequently encountered in the study of the origin of judged newly formed lymph vessels in polypoid implants since non-injected lymph vessels, when collapsed, cannot be detected. $\times 25$.

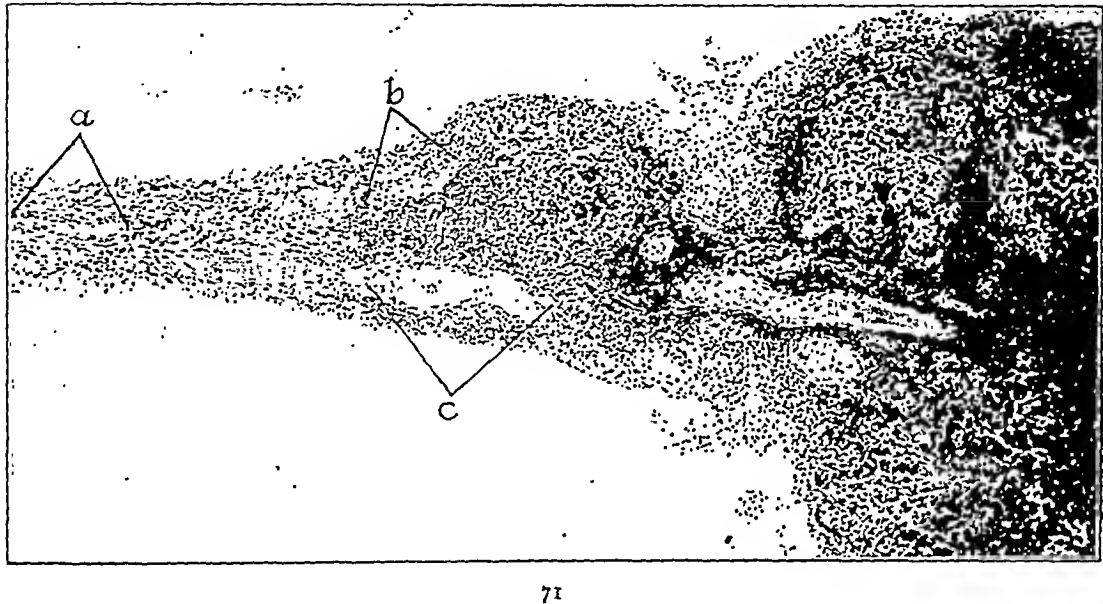
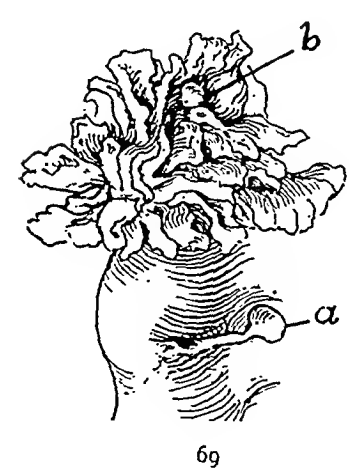


PLATE 98

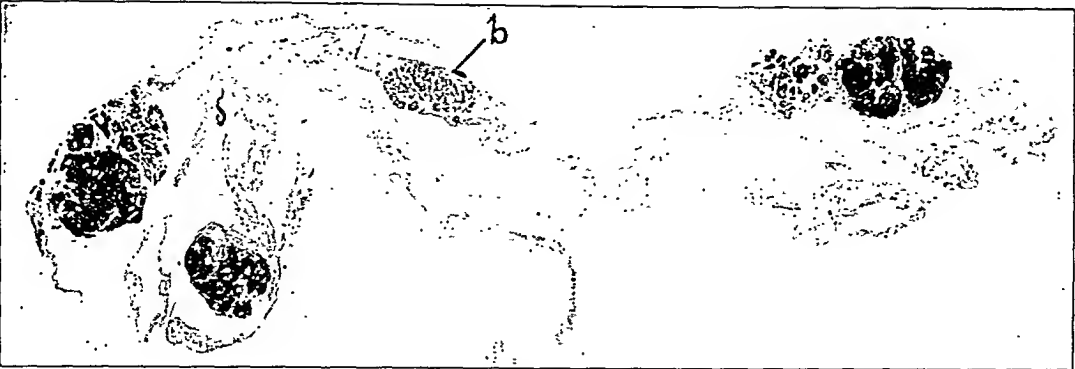
FIG. 73. Cobweb-like adhesions with metastatic carcinomas enmeshed in them like flies in a spider's web, from a patient (Case 8) with adenocarcinoma of both ovaries and a peritoneal carcinomatosis. These adhesions, which extended from the right tube to the suspensory ligament of the ovary, were carefully removed at the onset of the operation and immediately placed in formalin. The implants, shown here, are of nearly the same size and therefore may be of approximately the same age. However, in other sections of these adhesions all stages in the pathogenesis of these implants are encountered. $\times 5$.

FIG. 74. An early implantation of cancer cells in the adhesions shown in the preceding microphotograph and from the same series of sections. A growing clump of cancer cells is covered by granulation tissue which has arched over them. $\times 54$.

FIG. 75. A clump of viable appearing cancer cells caught in a loop of a strand of granulation tissue, also from the same series of sections as the preceding ones. $\times 130$.

FIG. 76. A very early stage in the implantation of cancer cells in a loop of granulation tissue similar to, but more vascular than, the preceding tissue. This stage is earlier than the one shown in Figure 74, also from the same series of sections. Cells from the granulation tissue beneath the carcinoma have invaded the fibrin holding the latter in place (compare with Figs. 36 and 37 showing a similar stage in the implantation of cancer cells on the tubal mucosa). $\times 130$.

FIG. 77. A judged early stage in the implantation of cancer cells on the mucosa of the ampulla of the right tube of Case 8. This area corresponds to the one marked by pointer "c" in Figure 85, from a nearby section in this series. Here the epithelium is lacking and viable appearing cancer cells are covered by fibrin which contains wandering cells from the sub-epithelial tissues of the mucosa. Compare with the implantation on serosal adhesions shown in the preceding microphotograph and also with Figures 36 and 37. The process apparently is the same in all four situations. Carcinoma once established in the tubal mucosa would be in close proximity to the mucosal lymph vessels and might easily invade these vessels and thus spread in the lymphatic circulation of the tube. $\times 130$.



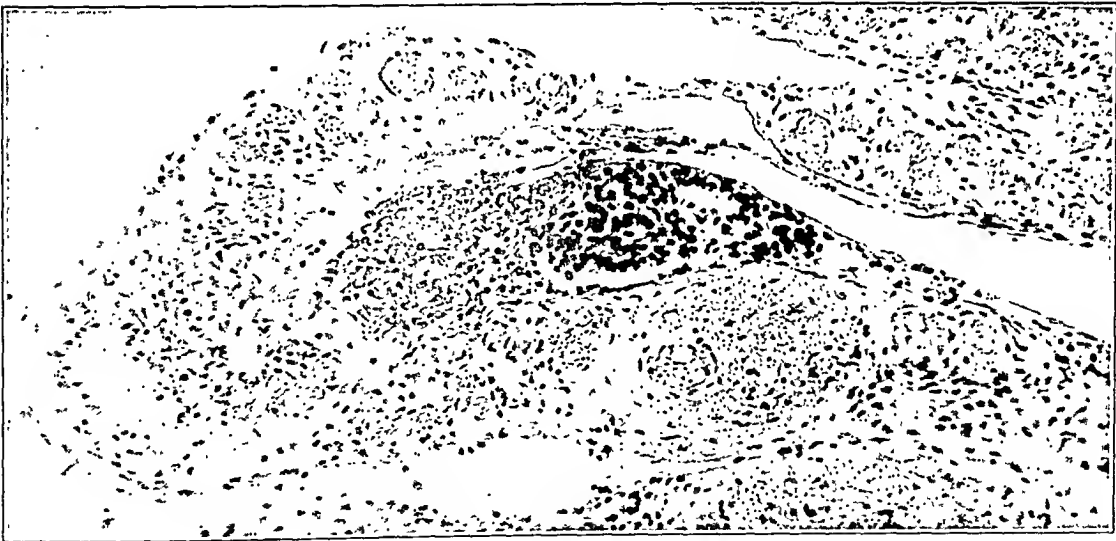
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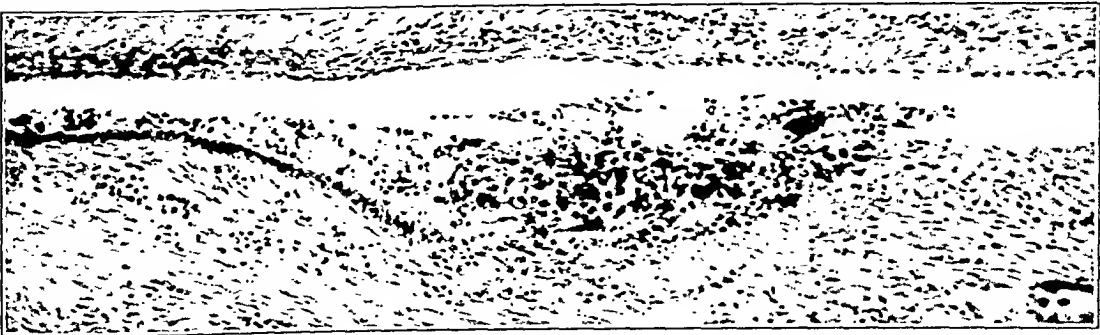
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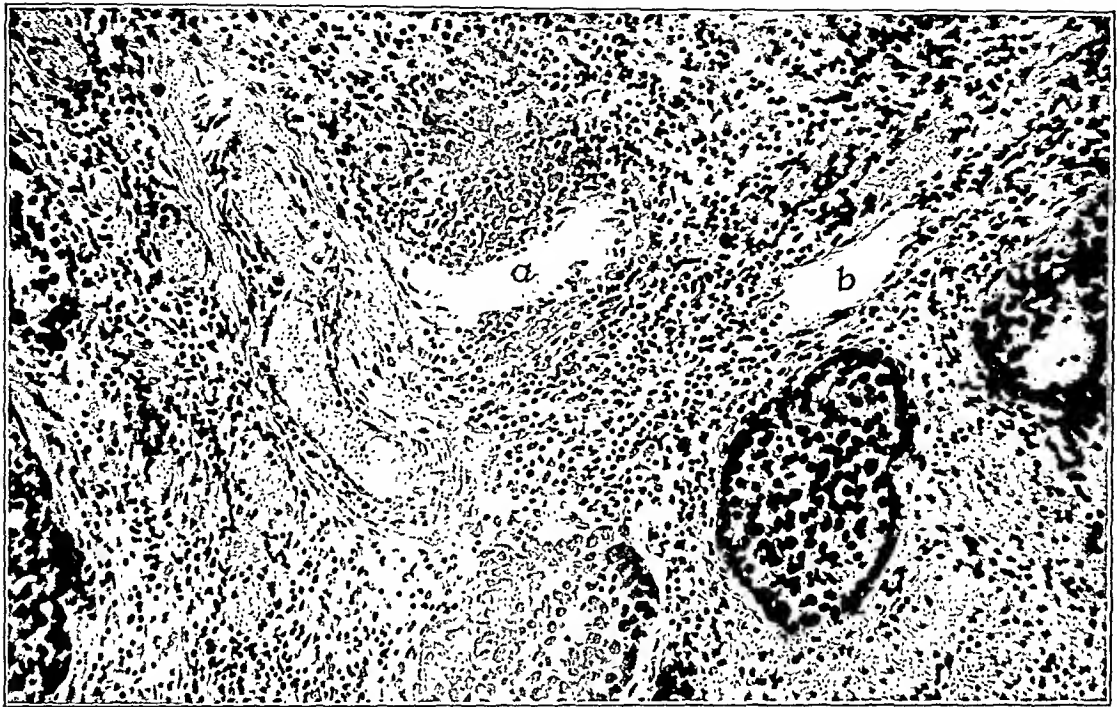
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PLATE 99

FIG. 78. A portion of a large implant in the adhesions shown in Figure 73, from another section of this series. Two judged lymph vessels or portions of the same vessel are marked "a" and "b." They can be easily distinguished from the blood vessels since the latter are filled with blood. It is impossible to determine whether or not carcinoma has invaded any of the lymph vessels in these adhesions. Some of the lymph vessels may have been invaded by carcinoma. $\times 130$.

FIGS. 79 and 80. Mature portions of the adhesions pictured in the preceding microphotographs showing easily recognized newly formed blood vessels and spaces "a" and "b" lined by endothelium-like cells without blood in their lumens. They are probably lymph vessels. Their continuity with preexisting lymph vessels of the tube or suspensory ligament of the ovary cannot be established since the adhesions were removed at the beginning of the operation. $\times 54$.

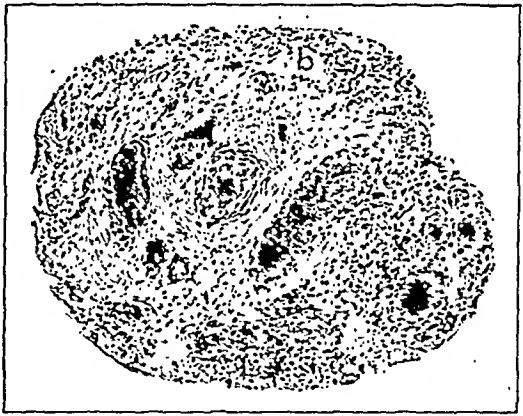
FIG. 81. A portion of a strand of newly formed tissue, similar to the adhesions just pictured, which has arched over the surface of the left tube, from the same patient. This strand contains newly formed blood vessels which are accompanied by a newly formed lymph vessel. The origin of the latter from a preexisting lymph vessel of the tubal wall can be seen plainly in this attachment of the strand to the serosa of the tube. The lymph vessel, shown above, extends the entire length of the strand and is continuous with a similar vessel in the attachment of the other end of the strand to the serosa of the tube (see Figs. 127 and 128 of a previous paper¹⁰). Carcinoma is not present in this newly formed tissue which forms the stroma of many implants. If it had been present it might have invaded the nearby lymph vessels. If it had been present in the preexisting lymph vessels of the tube beneath the attachment of the newly formed tissue it might have spread into the newly formed lymph vessels. This phenomenon has been demonstrated by the writer (see Figs. 129 to 131 inclusive of a previous paper¹⁰). $\times 130$.



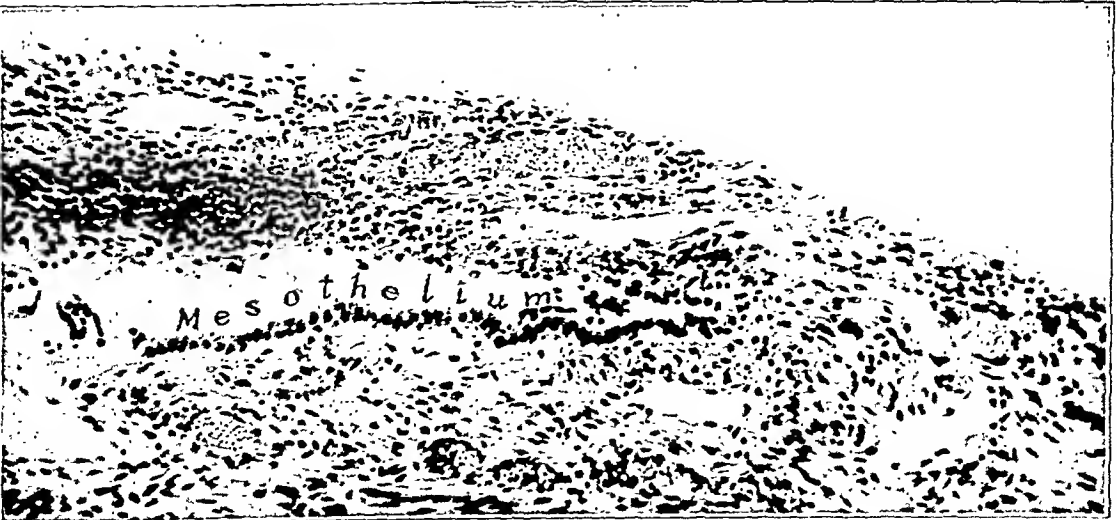
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PLATE 100

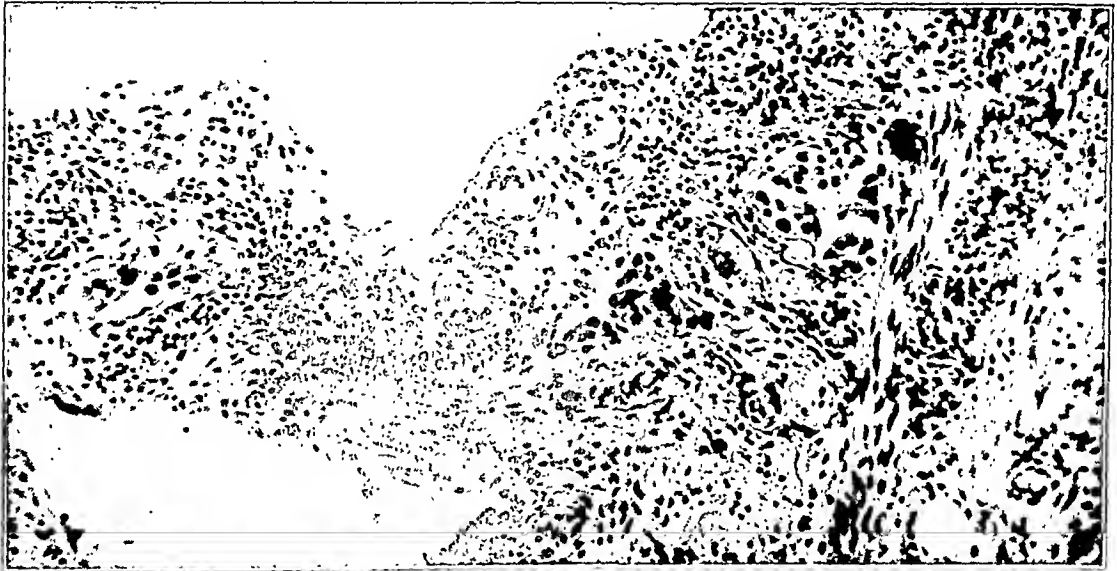
FIG. 82. A portion of the left mesosalpinx showing a strand of newly formed tissue which has arched over its surface similar to the strand shown in Figure 81, from the same patient. Both attachments of this strand to the serosa of the mesosalpinx appear in this section. Lymph vessels (possibly one vessel) are easily discernible (under higher magnification) throughout the entire length of the strand just as they are discernible in the strand in the preceding microphotograph. Cancer cells are present in the lumen of this vessel in all portions of the strand. I am unable to determine whether the growth of the carcinoma in the lymphatics is continuous throughout its entire length or is both continuous and interrupted (embolic). In the field shown in this microphotograph carcinoma is present in only the newly formed lymph vessel of the strand and in the preexisting lymph vessel of the subserosa from which the newly formed vessel arose (see the following microphotograph). However, in other portions of this section carcinoma is present both in large lymph vessels of the mesosalpinx and in its subserosal lymphatics. $\times 10$.

FIG. 83. Higher magnification of the right-hand portion of the strand of newly formed tissue and the mesosalpinx from which it arose, shown in the preceding microphotograph. Carcinoma is present in both the lumen of the newly formed lymph vessel and the lumen of the preexisting lymph vessel of the subserosa. The study of other sections in the series demonstrated that both the lymph vessel and the carcinoma in it in the strand of newly formed tissue are continuous with the lymph vessel and the carcinoma in the subserosa. Since carcinoma is present in both the deep and the subserosal lymph vessels of other portions of the mesosalpinx in this section and in other sections of the series, it is natural to assume that the carcinoma in the lymph vessel of the newly formed tissue reached its present situation by either lymphatic permeation or metastasis from the growth in the preexisting lymph vessels of the mesosalpinx (compare with Figs. 95 and 96). This field is an excellent example of peritoneal carcinomatosis of lymphatic origin. $\times 130$.

FIG. 84. Higher magnification of the loop of the strand of newly formed tissue indicated by "a" in Figure 82, from a nearby section in this series. This print is mounted at right angles to the one shown in Figure 82. The carcinoma in the upper arm of the loop is in a lymph vessel and is continuous with the carcinoma shown in the preceding microphotograph. The three clumps of judged cancer cells, below the carcinoma filled lymph vessel, are situated in the angle of a loop of newly formed tissue, similar to those shown in Figure 75. In spite of a careful study of the sections covering this field we were unable to establish a continuity between the carcinoma in the lymph vessel of the loop and that in the angle of the loop. I am not sure whether the clump of cells marked "a" is composed of cancer or mesothelial cells. Note that their nuclei are not as large as those of the cells in the clumps above them. $\times 130$.



82



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84

PLATE 101

FIG. 85. Longitudinal section of the distal end of the right tube and the ovarian carcinoma beneath it (Case 8). The tube is stretched over the carcinoma which has grown ("a") over the surface of the elongated lower lip of the fimbriae. A metastasis ("b") with the histological structure of a polypoid implant has arisen from the fimbriae representing the upper lip of the abdominal ostium of the tube. From its situation it must have arisen on the surface of a portion of the fimbriae covered with epithelium. The judged early implantation of cancer cells on the ampullar mucosa of Figure 77 is situated in a nearby section of this series in an area corresponding to that indicated by "c." $\times 5$.

FIGS. 86 and 87. Higher magnification of implant "b" of the preceding illustration and also of implant "b" of Figure 73. They both have a similar structure and probably a like pathogenesis. Compare with metastases of judged lymphatic origin shown in Figures 90 and 91. $\times 25$.

FIG. 88. Longitudinal section of the distal end of the left tube including its fimbriae proper, the ovarian fimbriae at the right and a portion of the mesosalpinx. Even under this low magnification the metastases "a" in mucosal folds of the fimbriae do not resemble the one marked "b" in Figure 85, from the same patient. Carcinoma is present in lymph vessels of the fimbrial mucosa and of the mesosalpinx. For a higher magnification of the two mucosal tumors, marked "a," see Figure 90. A patch of endometriosis on the mesosalpinx is marked "End." $\times 5$.

FIG. 89. A section similar to the preceding one and from the same series of sections. A mucosal fold of the ovarian fimbriae, to the right, is greatly enlarged and distorted by the tumor (compare with Fig. 22). Carcinoma can be seen under higher magnification in the lymph vessels of the mucosal folds. The pathogenesis of the mucosal carcinomas shown in the last two microphotographs was judged to be by permeation and metastasis from the ovarian tumor through the lymph vessels. $\times 5$.



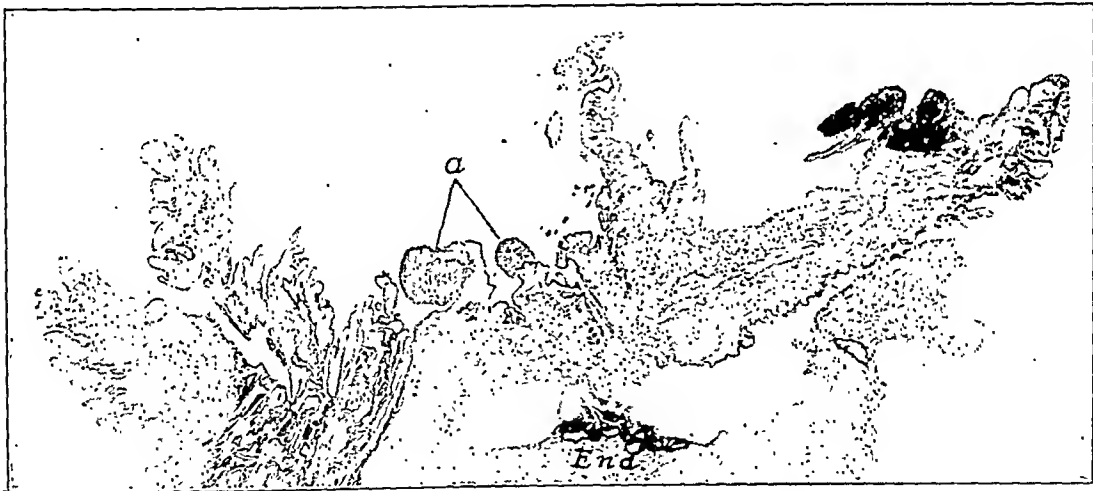
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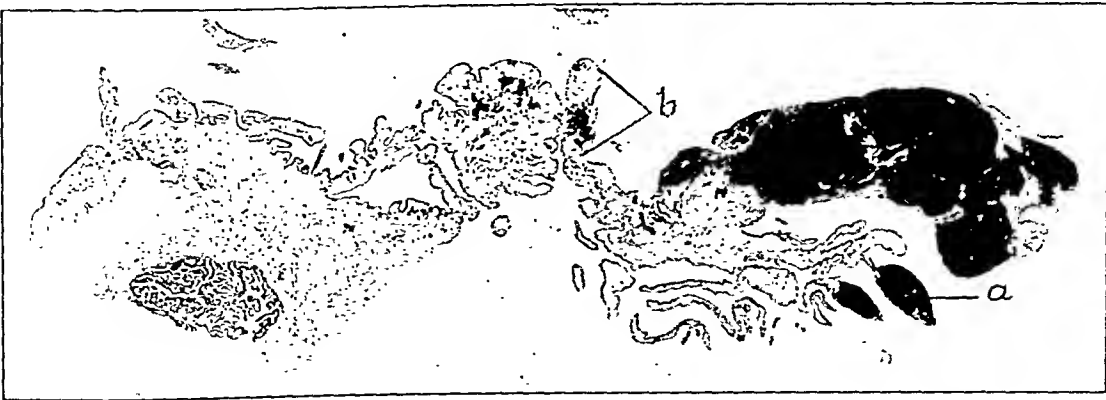
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FIG. 90. Higher magnification of the two mucosal folds marked by pointer "a" in Figure 88. Carcinoma is present in lymph vessels in the base of the first mucosal fold. The distal portion of both folds appears to have been distended by a growth which has reached and invaded their tissues from within. Compare with the metastases shown in Figures 66 and 70 where the metastatic tumors consist of the growth of cancer cells in newly formed tissue arising on the surface of a mucosal fold. $\times 25$.

FIG. 91. Higher magnification of the carcinoma in the mucosal fold marked "a" in Figure 89. In some ways the condition shown here resembles a pedunculated polypoid implant. The apparent pedicle is a non-distended portion of a mucosal fold, as in the two folds in the preceding microphotograph, and does not consist of newly formed tissue like the pedicles of the mucosal implants shown in Figures 67 and 68. $\times 25$.

FIG. 92. Carcinoma in a mucosal fold of the ampulla of the left tube, the fimbriae of which are shown in the four preceding microphotographs. Here the fold is distended by carcinoma which has invaded it from within and which apparently has grown in lymphatics. Carcinoma has even forced its way from a lymphatic through the overlying epithelium into the lumen of the tube (see "a"). This phenomenon is not of infrequent occurrence in situations where the mucosal lymph vessels are filled with a growing carcinoma (see Figs. 16 and 17). The anatomical basis for such a phenomenon may be seen in Figure 8. Carcinoma is present in lymph vessels in all portions of this tube including its fimbriae and in the mesosalpinx. Since no portal of entry for the carcinoma into the lymphatic circulation was discovered other than the ovarian tumor it may be reasonably assumed that the tumors of this tube were secondary to the ovarian by permeation and metastases through the lymph vessels. $\times 54$.

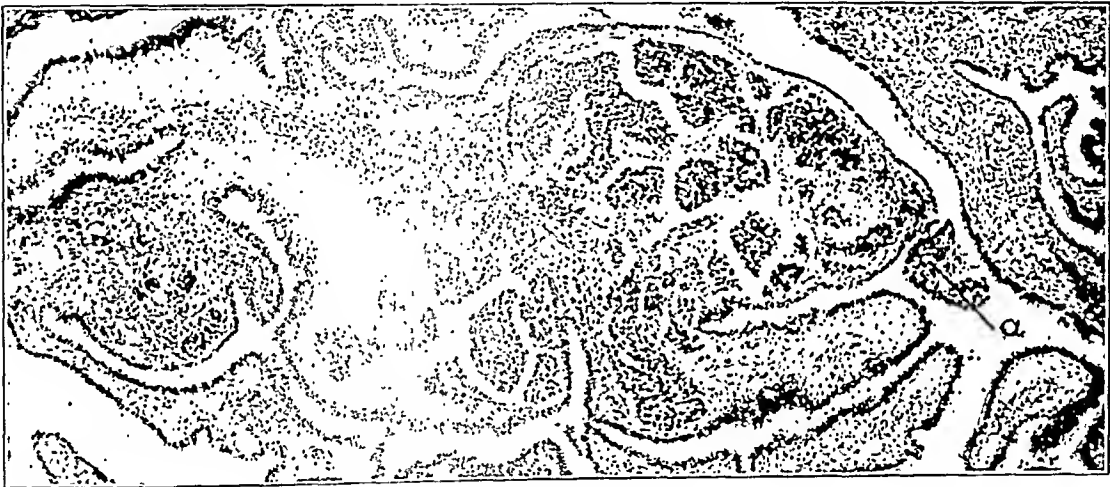
FIG. 93. Cancer cells ("a") becoming embedded in granulation tissue which has developed on the surface of a mucosal fold near the two folds marked "a" in Figure 88, from another section in this series. This granulation tissue is in the form of a polyp with a long slender vascular pedicle attached to a fold, the tip of which is shown at the extreme left of this microphotograph. Lymph vessels can be seen accompanying the blood vessels in the fold beneath the attachment of the pedicle (not shown in this microphotograph). The phenomenon shown here represents a stage in the implantation of cancer cells in granulation tissue arising from tubal fimbriae earlier than that shown in Figure 65. Carcinoma in a lymph vessel of an adjacent fold is marked "b." Carcinoma has developed in the fimbrial mucosa of this tube in two ways: one through lymph channels from the primary ovarian carcinoma; and the other by cancer cells escaping into the peritoneal cavity from the ovarian carcinoma and becoming implanted in granulation tissue which has developed on the surface of the fimbriae. Another implantation of carcinoma, similar to this one, was found in this specimen. $\times 54$.



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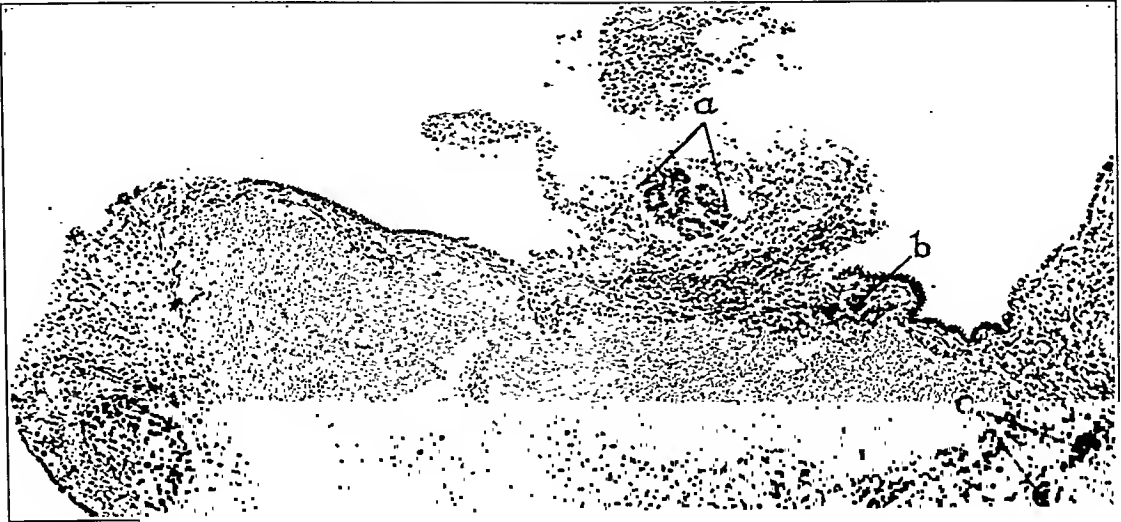


93

FIG. 94. A portion of the mucosal fold, marked "b" in Figure 89, but from another section in this series, with a sessile polypoid mass of early granulation tissue on its surface. Clumps of cancer cells ("a") are in a space in this tissue which resembles the lumen of a lymph vessel. Carcinomas "b" and "c" are shown in preexisting lymph vessels of the mucosal fold. $\times 54$.

FIG. 95. Higher magnification of the granulation tissue and a portion of the mucosal fold beneath it shown in the preceding microphotograph, from a nearby section in this series. The carcinoma in a judged newly formed lymph vessel in the granulation tissue of the previous section is also shown here. A small lymph vessel below a blood vessel is marked by "b." The preexisting lymph vessel with carcinoma in its lumen below the base of the granulation tissue is shown in the series of sections to be continuous with mucosal lymph vessel "b" of the preceding section. $\times 130$.

FIG. 96. The same granulation tissue shown in the preceding microphotographs, from a nearby section in this series. In this section the newly formed lymph vessel ("a"), which contains carcinoma in the previous sections, is shown to be continuous with the preexisting lymph vessel ("b") of the mucosa beneath the granulation tissue. It is evident, by comparing the last three microphotographs, that the carcinoma in a preexisting mucosal lymph vessel, in this instance, has spread into the newly formed lymph vessel, derived from it, in the granulation tissue. Compare with Figure 83 where the same phenomenon has occurred in granulation tissue arising on the serosa. I believe that carcinoma "c" is also in a lymph vessel. Since carcinoma frequently becomes implanted in granulation tissue it must also be evident that the carcinoma of the implant may invade a nearby newly formed lymph vessel, if the latter is present, and then spread to the preexisting lymphatics of the tubal mucosa. The implanted carcinoma may be small and not recognized as such. It is also true that carcinoma in a lymph vessel similar to the carcinoma shown here may penetrate the wall of the vessel and invade the tissue about it. Although there is no evidence of implanted carcinoma in this granulation tissue, it is present in granulation tissue in other situations of the fimbrial mucosa in this case (see Fig. 93). Eventually cancer cells might have become implanted in this granulation tissue if the operation had been deferred. Therefore, even if carcinoma in the stroma of a metastasis on the tubal mucosa can be shown to be continuous with carcinoma in a newly formed lymph vessel, it may be impossible to ascertain whether implanted carcinoma had invaded the lymph vessel, or carcinoma in the lymph vessel had invaded the tissue about it. $\times 130$.



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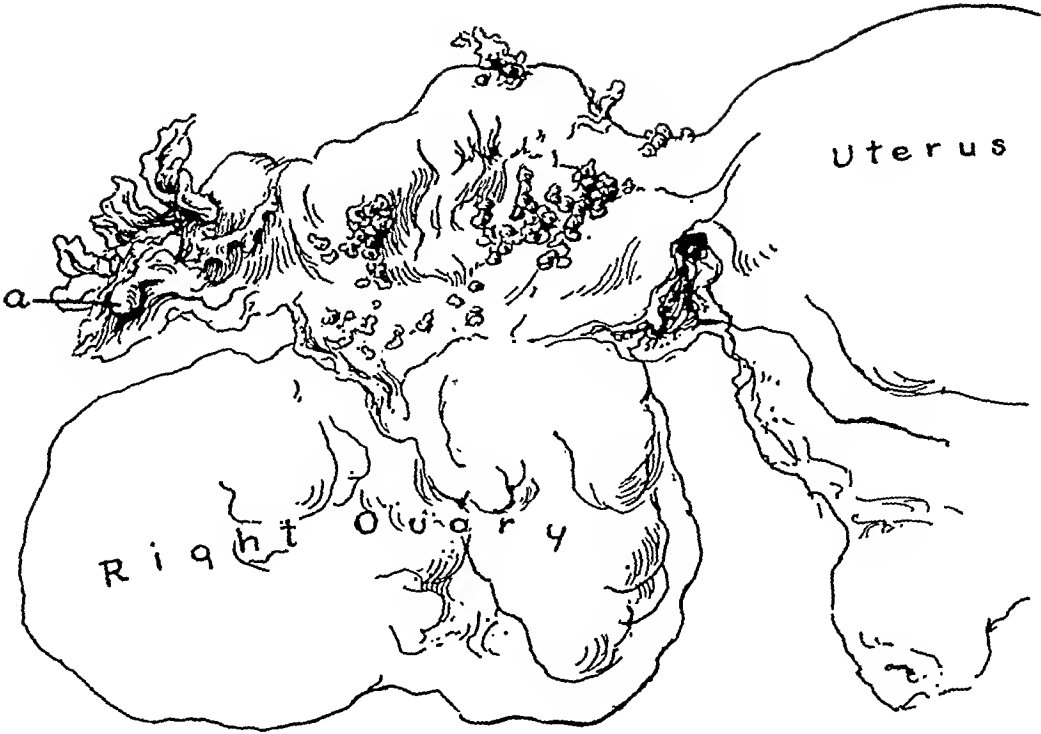
FIG. 97. Right ovary, tube and a portion of the uterus from a patient (Case 9) with carcinoma of both ovaries and an associated peritoneal carcinomatosis. Peritoneal implants, here, can be seen on the surface of the tube and mesosalpinx. Similar metastases were found on the surfaces of many other pelvic structures, including the sigmoid with its epiploic appendages (see Fig. 83 of previous paper¹⁰). At the operation the ovarian tumor was found to be adherent to the posterior surface of the uterus and had invaded that organ in this situation. A small nodule ("a") similar to the fimbrial implants shown in Figures 63 and 69 is attached to the tips of the mucosal folds of the fimbriae which appear to be fused at the base of the nodule. The left ovarian tumor was similar but smaller than the right. The left tube appeared normal except for a few implants on its serosa. Its fimbriae were grossly and histologically normal. Clumps of cancer cells, similar to those found in the ascitic fluid, were present in the lumens of both tubes and were much more numerous in the left tube (see Figs. 108, 109 and 112). Natural size.

FIG. 98. A very early implantation of cancer cells on the surface of the tube shown in the preceding illustration. The sections of the tube covering this area are sufficiently close together to demonstrate that the carcinoma in this situation is isolated and does not represent the advancing portion of a large growth. $\times 54$.

FIG. 99. Two early implantations of cancer cells ("a") in polypoid granulation tissue arising on the serosa of the tube shown in the preceding microphotograph. $\times 54$.

FIG. 100. A portion of a mature polypoid implant attached to the serosa of the same tube as the preceding ones. The condition, shown here, presents a stage in the life history of this type of implant later than that illustrated in the preceding microphotograph. $\times 54$.

FIG. 101. A cluster of implants of different ages on the serosa of the tube shown in the preceding microphotographs. To the right of the center is a sessile polypoid metastatic tumor which I believe is of implantation origin and well may represent a later stage of a condition similar to that shown in Figure 98. All stages in the development of implantation metastases, histologically similar to this one, were found on an epiploic appendage from this patient (see case report). Carcinoma is present in judged lymph vessels ("a") of the tubal wall and could well have come from the spread of the carcinoma above them. This is not an infrequent phenomenon in patients with peritoneal carcinomatosis. Attached to the upper surface of the mature sessile implant is a polypoid mass of fibrin with a clump of cancer cells ("b") enmeshed in its tip. The replacement of this fibrin by granulation tissue would give rise to a condition similar to the early polypoid granulation tissue at the left with carcinoma ("c") embedded in it. Beneath the latter is a still later stage in the life history of these implants a little earlier than the one in Figure 100. A portion of a sessile polypoid implant, granulation tissue stage, appears at the extreme right-hand portion of the microphotograph. Compare with Figure 107 showing a cluster of judged similar implants, also of different ages, on the fimbrial mucosa of the same tube. $\times 54$.



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100



101

FIG. 102. Longitudinal section of the distal end of the right tube, shown in Figure 97, in a plane that includes the fimbriae with the nodule indicated by "a" of that illustration, a portion of the mesosalpinx, and the abdominal ostium ("b") of the tube cut obliquely. The nodule "a" of Figure 97 can be seen in the center of the fimbriae. It appears as a darkly stained multinodular rounded tumor, with a constricted base, attached to the tips of mucosal folds which are fused together. This tumor points upward and to the left. At the right of its base is another tumor of about the same size, caused by the fusion of the tips of the mucosal folds in this situation (see Fig. 107). The fimbrial mucosa at the left appears normal. Carcinoma is not present in its lymph vessels. The lymph vessels of the fimbrial folds at the right are injected with carcinoma (see also Fig. 105), which apparently comes from the extension of the growth in lymph vessels at the base of and between the folds. The latter lymph vessels, filled with carcinoma, can be detected, even under this low magnification, in the mucosa between the fimbrial folds just described and the fimbrial tumors. It would appear that the carcinoma in the fimbrial folds at the right and in the fimbrial tumors might be closely related. Small emboli of cancer cells can be seen, under higher magnification, in lymph vessels indicated by the pointers "c" and "d." A higher magnification of the mucosal fold, marked by the arrow, is shown in Figure 106. $\times 5$.

FIG. 103. Higher magnification of the pedunculated polypoid implant on the serosa of the mesosalpinx marked "a" in the preceding microphotograph. It is a very mature serosal implant with a long tortuous pedicle. Implants similar to this one are not infrequent in patients with peritoneal carcinomatosis. Cross sections of the pedicle appear above the body of the implant. Cancer cells are present in a judged lymph vessel ("a") of the pedicle. These cells could well come from the implant and represent the attempted spread of the carcinoma in the implant into the lymph vessels of the mesosalpinx. Carcinoma was not found in the lymph vessels of the mesosalpinx beneath this implant. Cancer cells ("b" and "c") apparently are becoming embedded in newly formed tissue which recently has arisen on the surface of the implant (compare with Fig. 98). In this manner implants frequently increase in size. $\times 54$.

FIG. 104. Higher magnification of a portion of the normal appearing fimbrial fold shown at the left in Figure 102. The pattern of the lymph vessels in this fold is well illustrated. $\times 54$.

FIG. 105. Higher magnification of the fimbrial folds shown at the right in Figure 102. The lymph vessels of these mucosal folds (compare with those in the preceding microphotograph) are filled with carcinoma as with an injection mass. Carcinoma is also present in lymph vessels of the mucosa at the base of and between the folds. Lymphatic metastases or permeation from the ovarian tumor, which in this case had invaded the lymph vessels of the ovary, would be an acceptable explanation for the carcinoma in this situation. The possibility of the origin of the carcinoma in the lymph vessels from the spread of the secondary mucosal tumors of the fimbriae shown in Figure 102 also must be considered (see Fig. 107). $\times 54$.



102



103



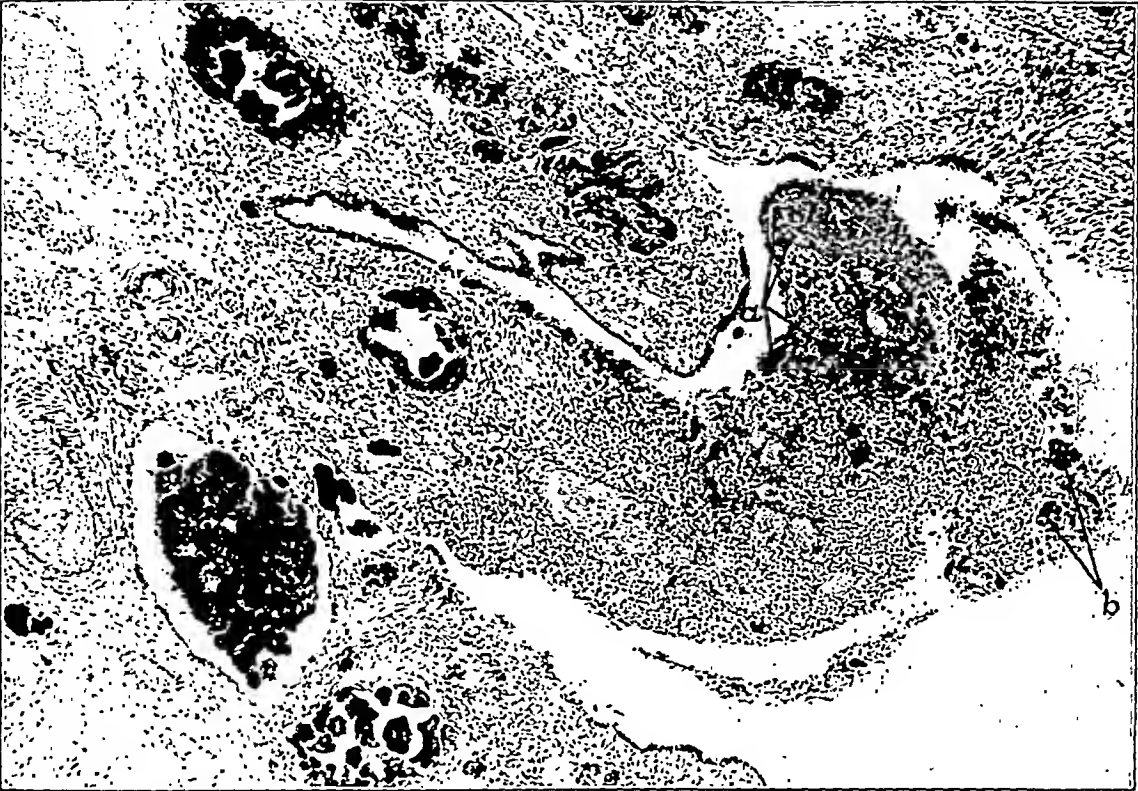
104



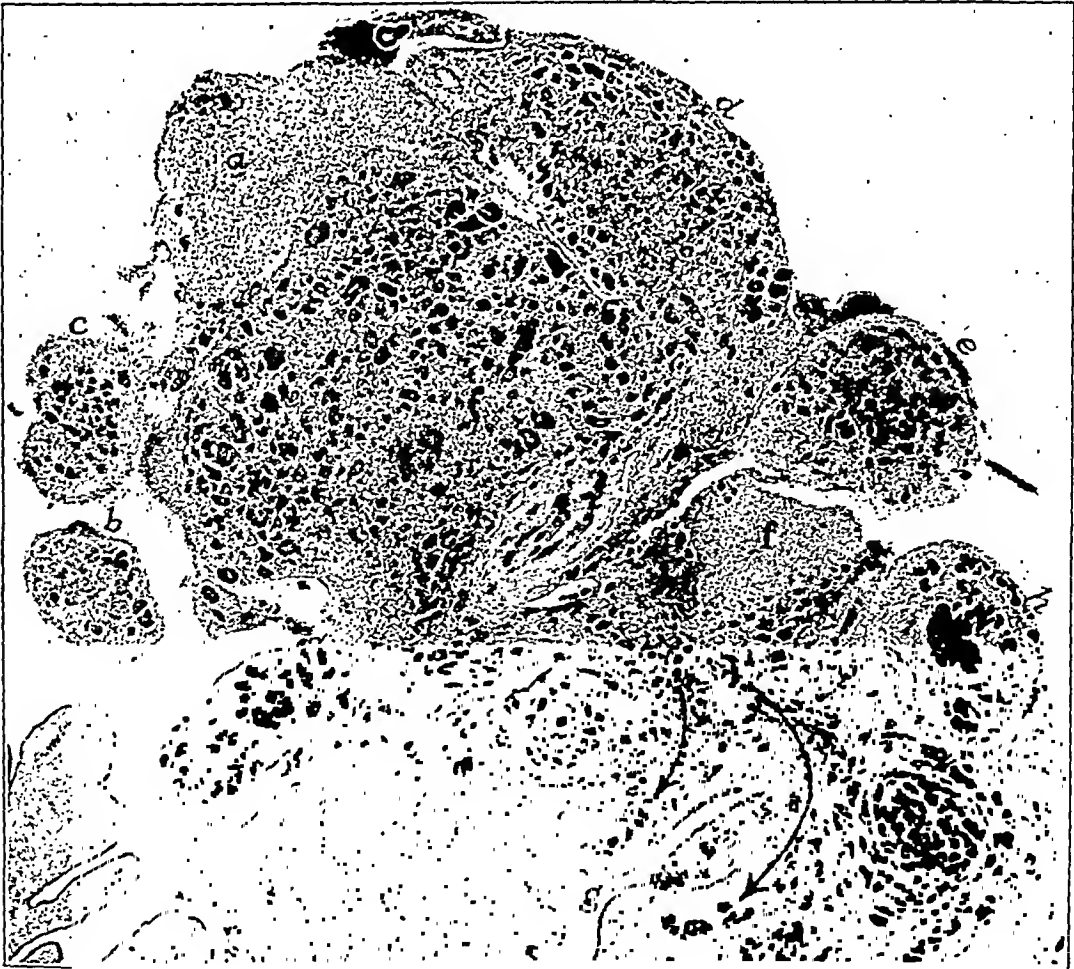
105

FIG. 106. Higher magnification of the mucosal fold of the fimbriae marked by the arrow in Figure 102. A polypoid mass of early granulation tissue has arisen from the tip of this fold. Clumps of cancer cells ("a") are partially or completely embedded in the distal portion of this tissue. Compare with similar conditions arising on the serosa of the same tube shown in Figures 99 and 101. Note the implantation of clumps of cancer cells ("b") in a younger portion of this tissue similar to that shown arising not only on the tubal serosa of this case (see Figs. 98 and 103) but also on the fimbrial mucosa of other cases (see Figs. 30, 47, 65 and 93). The cancer cells, just described, undoubtedly came from the ascitic fluid in which the fimbriae were immersed (see Fig. 108). The carcinoma in the lymph vessels of the base of this fold and in the adjacent fimbrial mucosa clearly antedates the cancer cells implanted in the granulation tissue of the fold. The carcinoma in the lymph vessels must have arisen by permeation or embolism either primarily from the ovarian growth or from the secondary mucosal neoplasms shown in Figure 102. Granulation tissue like this, arising on adjacent folds, would cause the fusion of the folds shown in the next microphotograph. $\times 54$.

FIG. 107. Higher magnification of the tumor or tumors attached to the fused tips of mucosal folds of the fimbriae shown in Figure 102. How may one account for the condition shown here other than by permeation or metastasis through the lymph vessels from the primary ovarian tumor? Above the center of the microphotograph and a little to the left is situated a multinodular conglomerate tumor which consists of polypoid outgrowths of judged newly formed tissue of various ages containing carcinoma (similar to those shown in Figs. 99, 100 and 101), which are either fused with or clustered about a central tumor. The amount of carcinoma in each outgrowth varies, as in the latter situations, with the age of the outgrowth. Outgrowth "a," which consists of very early granulation tissue, contains only a few small clumps of cancer cells implanted on its surface and embedded in it (compare with the preceding microphotograph). In the polypoid newly formed tissues, "b" and "c," which appear in cross section, the stroma not only is older than that in "a," but it contains a correspondingly greater amount of carcinoma (compare with Fig. 101). The amount of carcinoma in the polypoid outgrowth of newly formed tissue "d" is still greater than in the preceding ones (compare with Fig. 100). The conditions just described are similar to the various stages in the pathogenesis of polypoid implants on the serosa of patients with peritoneal carcinomatosis (see Fig. 101 showing a cluster of implants of various ages on the serosa of the same tube). In this section similar polypoid outgrowths are clustered about or fused with a central polypoid outgrowth similar but larger. This central tumor is attached by a constricted base or pedicle to the fused mucosal folds beneath it. Since the conglomerate tumor has grown by the fusion of probable multiple polypoid implants of various ages, the central and apparently oldest neoplasm in this situation, with a histological structure similar to that of the older tumors about it, also may be of implantation origin. Other sections in the series indicate that the carcinoma in the central tumor well may have invaded the fused mucosal folds beneath its constricted base (pedicle). This phase of the picture shown here is very similar to that illustrated in Figure 101 and also to that found in other serosal implantations of carcinoma. A further discussion of the conditions shown in this microphotograph appears in the report of this case (Case 9). $\times 25$.



106



107

Sampson

Implantation Carcinoma of Tubal Mucosa

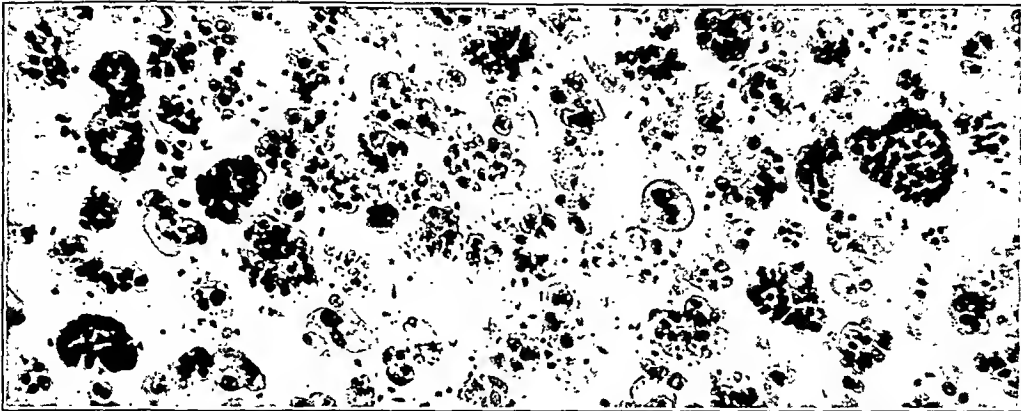
PLATE 107

FIG. 108. Section of the sediment from the centrifugalized ascitic fluid in which the fimbriae shown in the preceding microphotographs were immersed. Note the clumps of viable appearing cancer cells, which might become attached to and grow either on the tubal serosa or on the fimbrial mucosa if a suitable soil should arise in these locations (see Figs. 98, 101, 106 and 107).

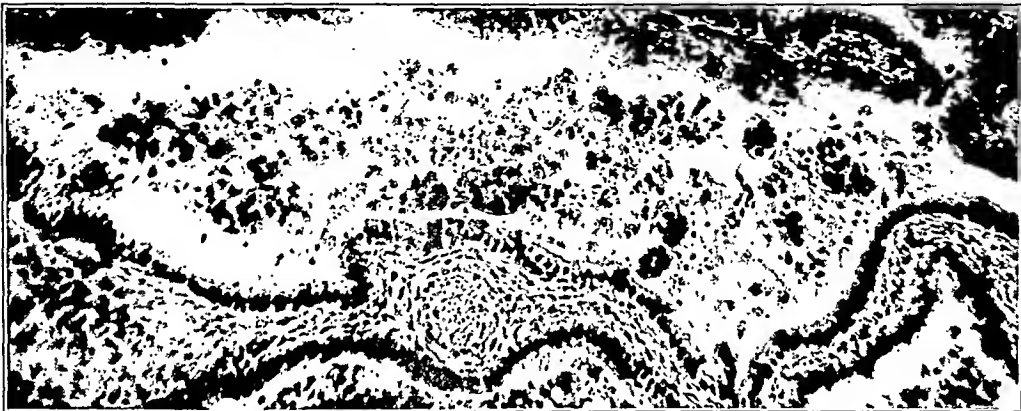
FIG. 109. A portion of a longitudinal section of the ampulla of the left tube near its abdominal ostium from the same patient as the cancer cells shown in the preceding microphotograph. The lumen of the entire ampulla of this tube contains cancer cells similar to those shown in the preceding microphotograph. The fimbriae of this tube are grossly and histologically normal. It is believed that cancer cells (similar to those shown in Fig. 108) entered the lumen of this tube through its abdominal ostium. Note that in this field the mucosal epithelium is intact and that there is no visible attempt on the part of the cancer cells to replace it. $\times 130$.

FIG. 110. A portion of the same section shown in the preceding microphotograph but nearer the isthmus of the tube. Here a change has taken place. The tubal epithelium not in contact with cancer cells is intact. On the other hand, cancer cells are growing and apparently attaching themselves to another portion of the mucosa by actually replacing the epithelium. $\times 130$.

FIG. 111. A field similar to the preceding one and from the same section. The replacement of mucosal epithelium by carcinoma is even more evident here than in the preceding microphotograph. It has been shown that the mucosa of the ampulla of the tube is abundantly supplied with lymph vessels. Judged lymph vessels are marked "a" and "b." The clump of cancer cells "c" well may be in a lymph vessel. I believe that the carcinoma in this vessel came from the secondary carcinoma of the tubal mucosa rather than from the ovarian tumor by lymphatic embolism or permeation. No evidence of the latter was detected in this tube. Carcinoma was not found in lymph vessels of other portions of this tube. On the other hand, in spite of the examination of several sections of this block, I was unable to demonstrate the invasion of this lymph vessel by the nearby growth of the mucosa. Since complete serial sections had not been made, this phenomenon, though present, could have been missed. $\times 130$.



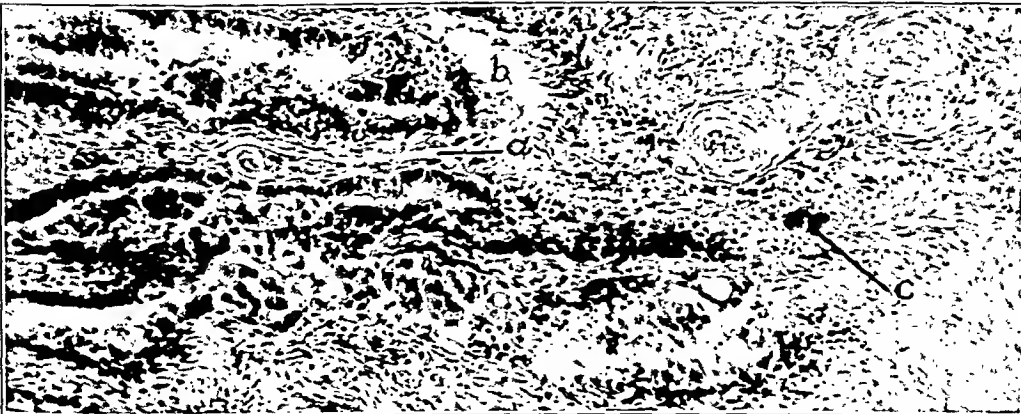
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109

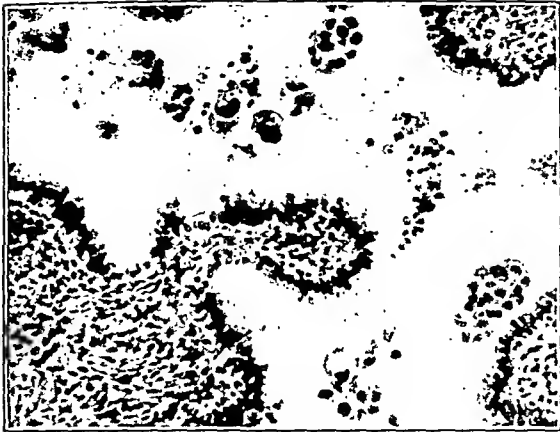


110

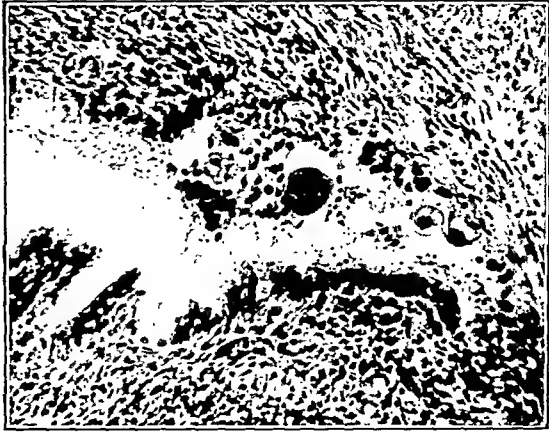


111

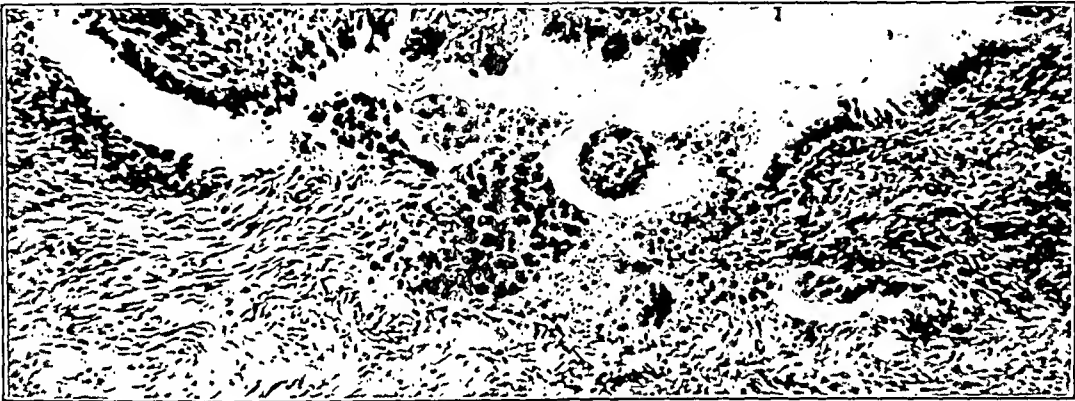
- FIG. 112. A portion of a longitudinal section of the ampulla of the right tube, the fimbriated end of which is shown in Figure 102. Cancer cells singly and in clumps, similar to those in the ascitic fluid (see Fig. 108), are present in all portions of the lumen of the ampulla of this tube. However they are not as numerous as those in the lumen of the opposite tube (see Fig. 109). $\times 130$.
- FIG. 113. A portion of the ampulla of the right tube near its isthmus, from the same series as the section shown in the preceding microphotograph. Here cancer cells are in contact with and may be actually growing on a portion of the mucosa lacking its epithelium. The etiology of this raw area of the surface of the mucosa is not evident. $\times 130$.
- FIG. 114. Another portion of the right tube, nearer the isthmus than that shown in the preceding microphotograph and from the same series. Here cancer cells have replaced the mucosal epithelium and have actually formed a tumor. The two areas with carcinoma replacing the tubal epithelium (shown in the last two microphotographs) are not continuous. The condition shown in the last field may well represent a later stage of that shown in the preceding one. Compare also with Figures 110 and 111 from the opposite tube of the same patient. A similar phenomenon apparently is present in the two tubes. $\times 130$.
- FIG. 115. Carcinoma of the tubal mucosa appears in the left half of this field (from the same series as the preceding section). From a study of other sections of this area it was judged that the carcinoma shown here is another portion of the growth pictured in the preceding microphotograph. I do not believe that this portion of the carcinoma is in a lymph vessel. However, carcinoma "a" well may be in a mucosal lymph vessel. $\times 130$.
- FIG. 116. A portion of the right tube near that shown in the preceding microphotograph. Carcinoma ("a") is shown in judged lymph vessels, probably two portions of the same vessel. Complete serial sections of this block had not been made. Nevertheless from the sections available I believe that probably the carcinoma shown in the judged lymph vessels ("a" of this and of the preceding microphotograph) may be continuous. Carcinoma was found in the lymph vessels of the mucosa of this tube only in the fimbriae (see Fig. 102) and in the ampulla, near the isthmus, shown here. Carcinoma was found in subserosal lymph vessels beneath some of the larger serosal implants of this tube (see Fig. 101 of this paper, and Figs. 85 and 89 of a previous paper¹⁰). I fully realize that the carcinoma which has replaced the ampullar epithelium, in Figure 114, may have reached this situation by metastasis from the ovarian carcinoma through the lymph vessels since the carcinoma in the ovary had invaded the lymph vessels of that organ. On the other hand, very strong circumstantial evidence has been presented indicating that cancer cells escaping from the ovarian tumor into the peritoneal cavity entered the lumens of both tubes, became implanted on their mucosa, and subsequently invaded the lymph vessels of the latter (see Figs. 108 to 115 inclusive). $\times 130$.



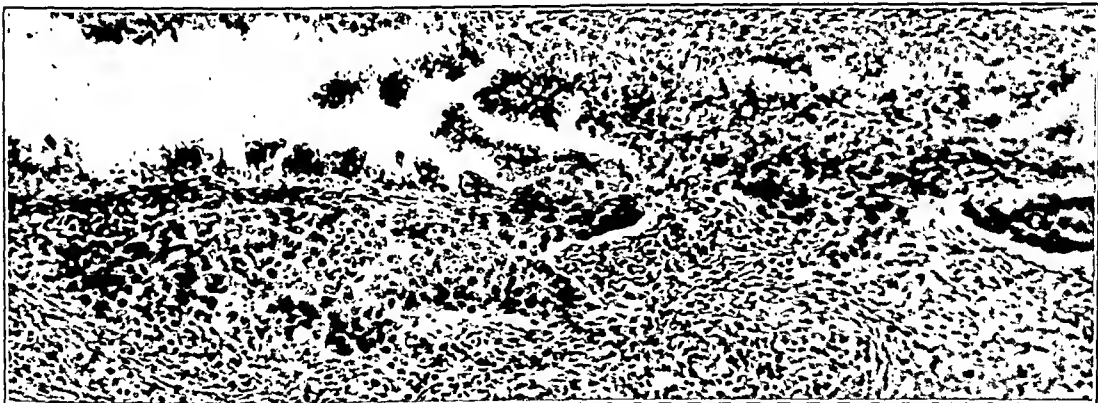
112



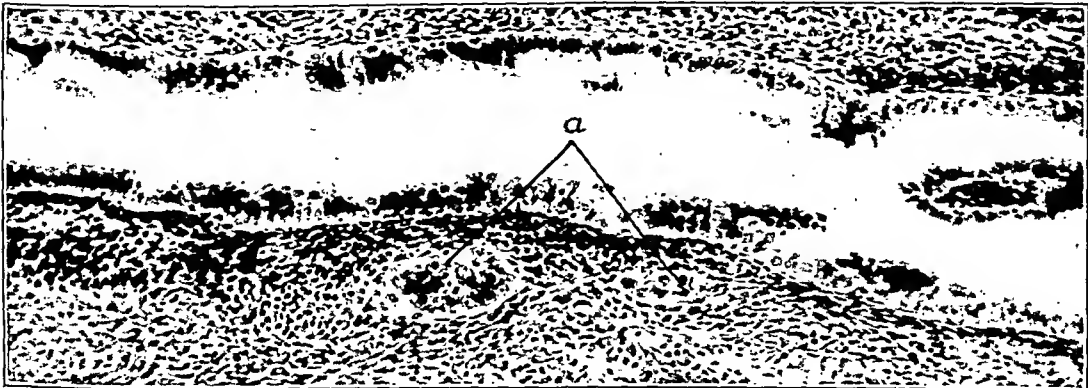
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114



115



116

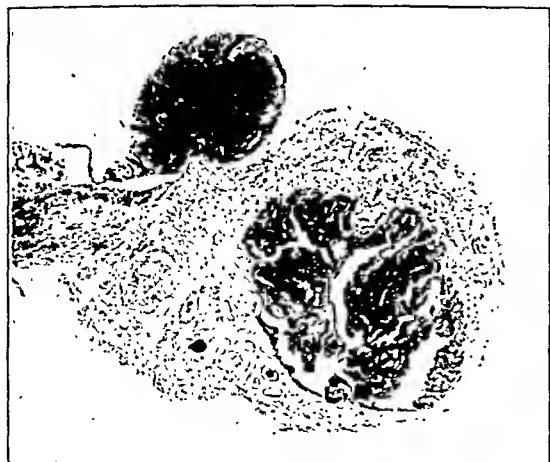
FIG. 117. A polypoid carcinomatous implant on the serosa of the tube from a patient with carcinoma of both ovaries and peritoneal carcinomatosis (Case 4 of a previous paper⁹ and Case 10 of the present paper). The implant is attached to the wall of the tube by a short vascular pedicle. $\times 5$.

FIG. 118. Two polypoid implantation-like metastases ("a" and "b") of the mucosa of the opposite tube (Case 10). The larger one is attached to the mucosa by a short vascular pedicle. The pedicle of the smaller and apparently younger implant, situated below the larger one, was missed in the few sections which were made of this tube. The various stages in the development of metastases like these, from cancer cells becoming implanted in granulation tissue arising on the surface of the mucosa of the tube, were not found in this case. However, it is demonstrated in other instances in this article (see Figs. 27, 28, 29, 46, 47, 65 and 68) and may have been missed in this case since the entire tube was not studied microscopically. Clumps of cancer cells are present in the lumens of both tubes but were not found in the lymphatics of either of the tubes or the ovaries. However, they were found in lymph vessels beneath implants on the parietal peritoneum (see Fig. 120 of previous paper⁹), and also in a lymph vessel in the peritoneum of the mesosalpinx beneath the pedicle of the polypoid implant shown in Figure 121 of this paper. $\times 5$.

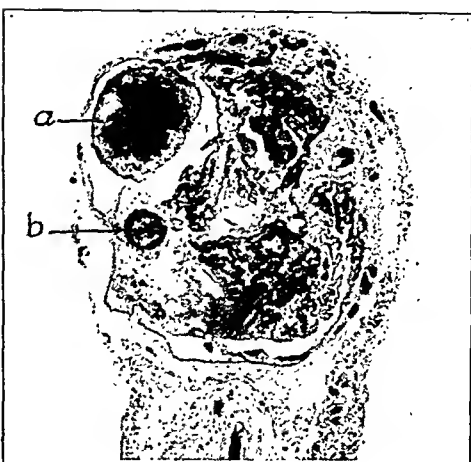
FIG. 119. A pedunculated polypoid implant on the serosa of the mesosalpinx of the tube shown in Figure 117. It is not only smaller but also apparently younger than the preceding serosal implant. Cancer cells in the peritoneal cavity may become implanted on recently newly formed tissue arising on the surface of such an implant. Carcinoma embedded in the implant may invade its stroma and reach its surface. $\times 25$.

FIG. 120. Higher magnification of the smaller metastasis on the tubal mucosa shown in Figure 118. Compare with the serosal implant shown in the preceding microphotograph. The histological structure of the two tumors is quite similar but not identical. The stroma of this metastasis is more compact and less vascular than that of the preceding one. It resembles more closely the stroma of the polypoid serosal and mucosal implants shown in Figures 122, 124, 128, 131 and 138. A portion of the lower pole of the larger metastasis of Figure 118 appears in the upper portion of this microphotograph. The carcinoma on the surface of the lower pole of this neoplasm may have arisen from the implantation of cancer cells on its surface or from the extension of the carcinoma in the neoplasm. Both of the metastases, shown here, have developed in polypoid newly formed tissue arising on the surface of the mucosa as in serosal implants. Carcinoma was not found in lymph vessels of any portion of the tube but was detected in one situation in the mesosalpinx (see the next microphotograph). I believe that all of the four metastases, just shown, had a similar origin. $\times 25$.

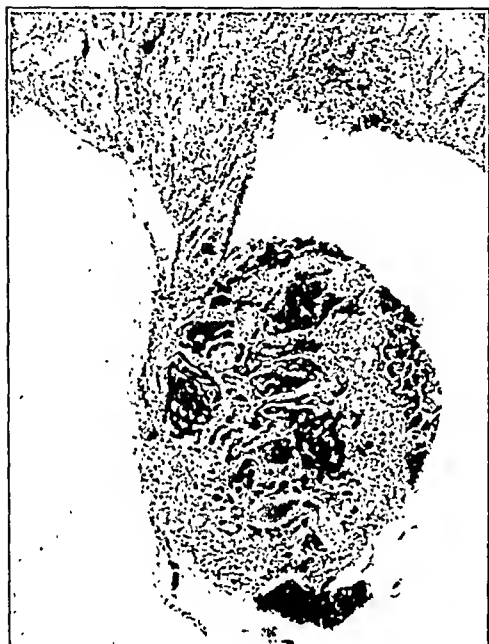
FIG. 121. A polypoid implant on the serosa of the mesosalpinx shown in Figure 119. It is larger and apparently older than the preceding serosal implant. Lymph vessels cannot be detected in its vascular pedicle. However, cancer cells ("a") are present in a judged lymph vessel of the serosa beneath the attachment of the pedicle of the implant. I believe that these cells probably came from the carcinoma in the implant above them rather than from the primary ovarian tumor by retrograde lymphatic metastasis. $\times 25$.



117



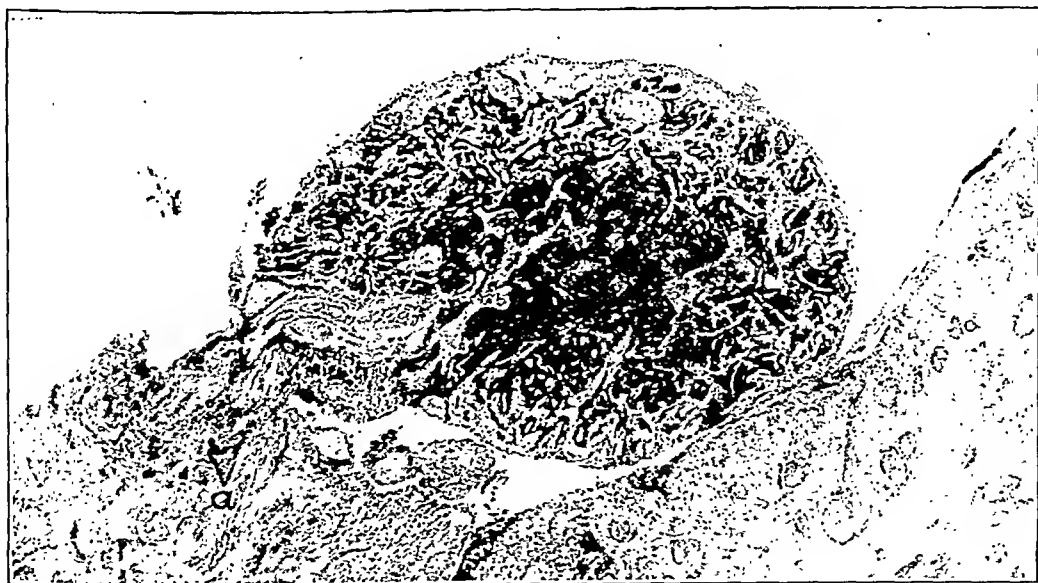
118



119



120



121

PLATE 110

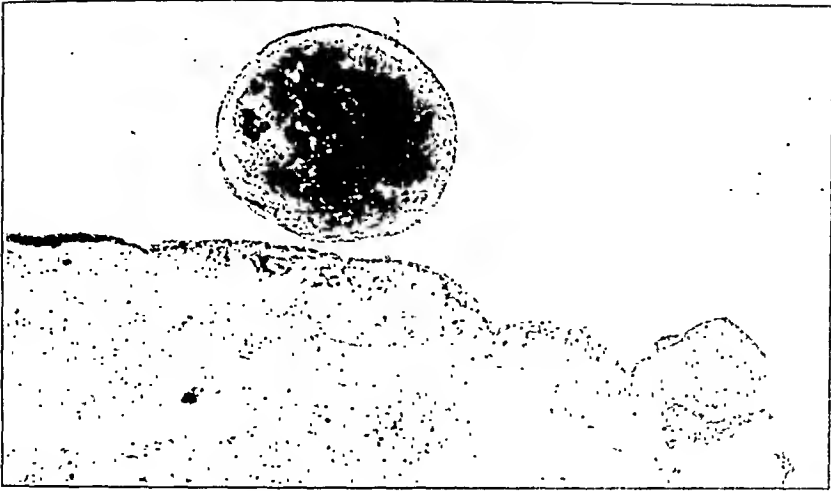
FIG. 122. Two mature polypoid carcinomatous implants on the serosa of an epiploic appendage from a patient (Case 11) with carcinoma of both ovaries and a peritoneal carcinomatosis. The larger implant is attached to the appendage by a slender vascular pedicle (see the next microphotograph). The smaller implant is pear-shaped and is attached by its small end to the appendage. The carcinoma in the implants had not invaded the preexisting tissues of the appendage. Carcinoma could not be found in the lymph vessels of this appendage. $\times 10$.

FIG. 123. A portion of the pedicle ("a") of the larger implant shown in the preceding microphotograph, at its attachment to the appendage. $\times 10$.

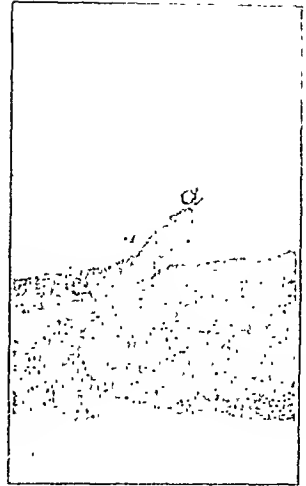
FIG. 124. Three mature polypoid implants on another epiploic appendage from the same patient as the preceding ones. The largest one is sessile: the growth in its base has invaded the appendage at "a." The second implant ("b") is attached to the appendage by a short pedicle. Carcinoma can be seen, under higher magnification, extending through the pedicle toward the appendage. However, the invasion of the appendage by the carcinoma in the pedicle could not be detected. A very small implant ("c") with a slender pedicle is shown, at the right of the second implant. Blood vessels in the appendage accompanied by lymph vessels with carcinoma in their lumens are marked "d." $\times 5$.

FIG. 125. The same implants, shown in the preceding microphotograph, from a section further on in this series. Carcinoma beneath the serosa of the appendage (see Fig. 127) is indicated by "a." This is very near the attachment of the pedicle of the second implant, see the preceding microphotograph. However, I was unable to detect its continuity with the growth in the implant above it. This might have been missed as the series of sections was not quite complete. Carcinoma is present in the serosa of the appendage at "b" and also in lymph vessels accompanying blood vessels (see the dark areas in the appendage above the serosa just mentioned). Sections further on in the series suggest that the carcinoma indicated by "a" may be continuous with that in the lymphatics in the other dark areas. $\times 5$.

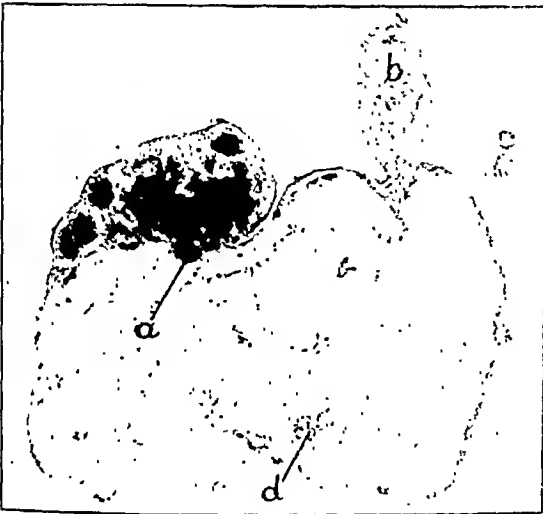
FIG. 126. Higher magnification of the blood vessels accompanied by lymph vessels with carcinoma in their lumens, marked "d" in Figure 124. A large lymph vessel with carcinoma in it is marked "a." Smaller vessels also with carcinoma in them are marked "b" and "c." Did the carcinoma in these vessels come primarily from the ovarian tumors through the ovarian lymph vessels and thence, in a roundabout manner, to the lymph vessels of the appendage, or did the carcinoma in the implants on the surface of the appendage invade the underlying lymph vessels? (See Figs. 128, 129 and 130.) $\times 130$.



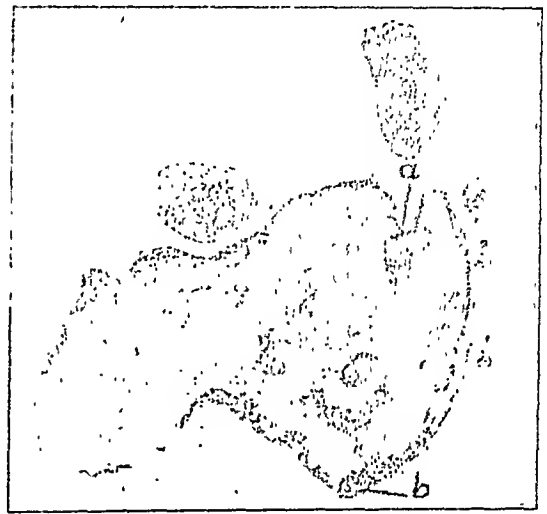
122



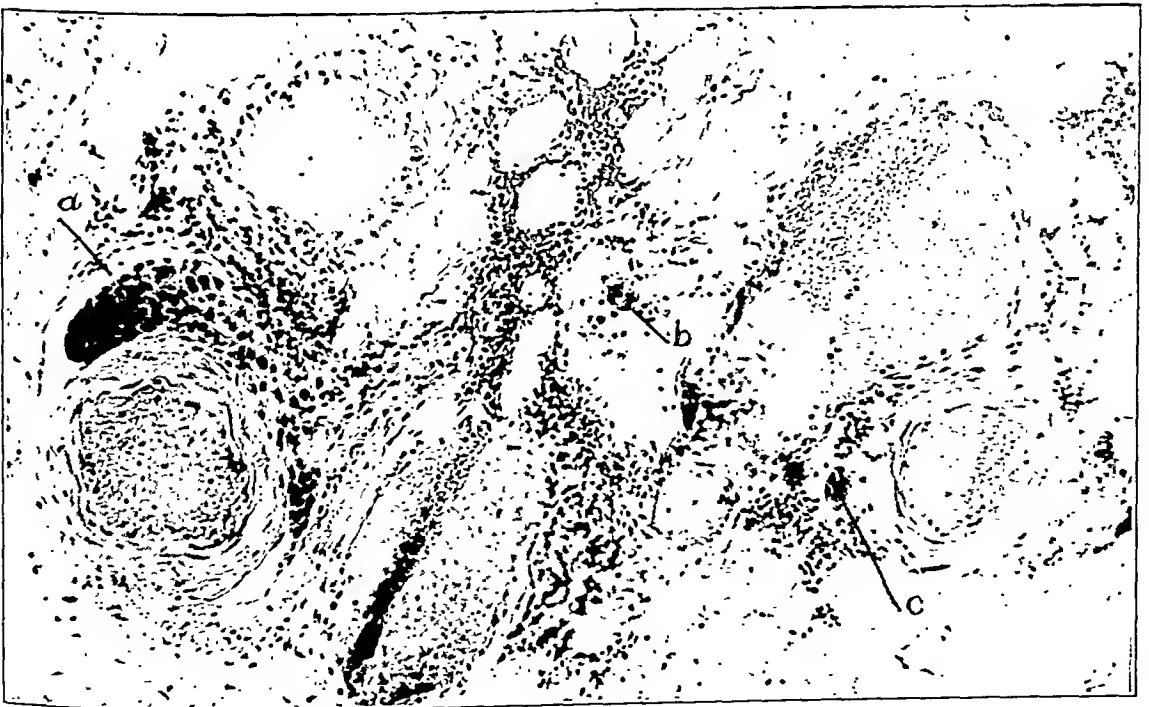
123



124



125



126

PLATE III

FIG. 127. Higher magnification of a portion of the second implant and the appendage beneath it shown in Figure 125. The lower portion of the body of the implant appears in the upper half of the microphotograph. The dark area "a" of the tissues of the appendage consists of carcinoma surrounded by dense fibrous tissue. Note that it is below the serosa. Carcinoma was not found either in the serosa or on its surface in this situation. As stated in the legend of Figure 125, the continuity of the carcinoma in this implant with that in the appendage below it was not demonstrated. $\times 25$.

FIG. 128. A large, pear-shaped, sessile polypoid implant and a portion of the appendage beneath it. This implant is attached to the appendage, near the area marked "b" in Figure 125. Note that carcinoma is present in the thickened serosa ("a") of the appendage and, I believe, is continuous with the growth in the implant above (see the next microphotograph). Also compare with the area, marked "a" of the preceding microphotograph, where the carcinoma is below the serosa. $\times 25$.

FIG. 129. The attachment of the implant, shown in the preceding microphotograph, to the epiploic appendage. The implant evidently was injured during the removal of the appendage and is partially torn from the latter. However, a portion of the implant is still attached to the serosa of the appendage in the bottom of an indentation of the appendage into which the base of the implant fitted. The implant is also still attached to the appendage in the upper left-hand portion of the field shown in this microphotograph. The carcinoma of the implant has invaded the epiploic appendage above and to the right of the blood vessel marked "a." $\times 25$.

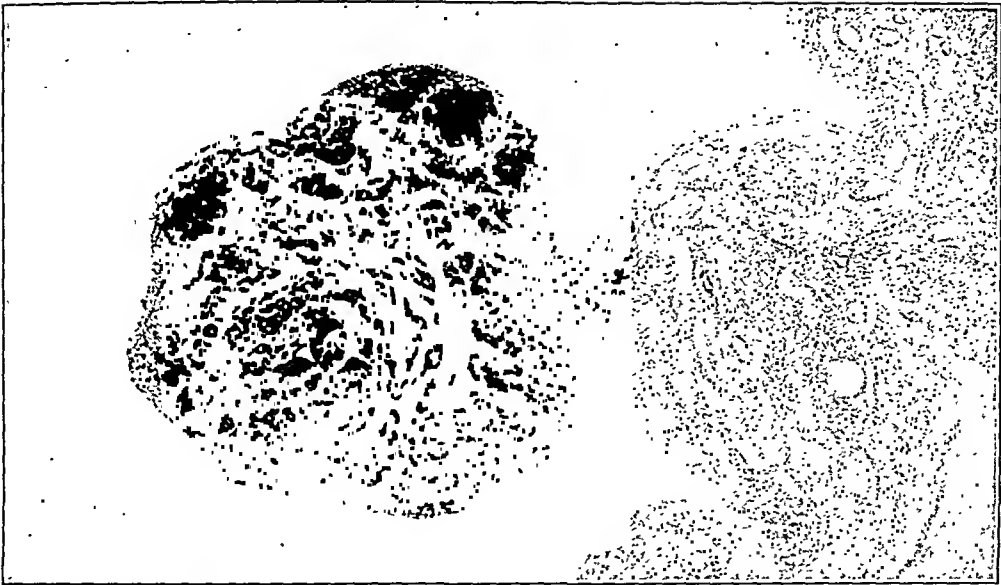
FIG. 130. A higher magnification of the area about the blood vessel "a" of the preceding microphotograph but from a section further on in this series. A blood vessel, a continuation of vessel "a" of the preceding section, can be followed into the deeper tissues of the appendage. This vessel is accompanied by a lymph vessel distended with carcinoma (see arrows) which evidently came from the growth situated about the upper end of the upper arrow. The carcinoma just mentioned is a continuation of the growth in the base of the implant at its attachment to the appendage shown in the preceding microphotograph. I am convinced the carcinoma in the lymph vessels of the appendage came from this source and not from the ovarian tumor by roundabout and retrograde lymphatic permeation or metastasis. Compare with Figure 122 in which an epiploic appendage had similar implants with no evidence of an extension of the carcinoma from the implants into the appendage and without carcinoma in its lymph vessels. $\times 54$.

PLATE 112

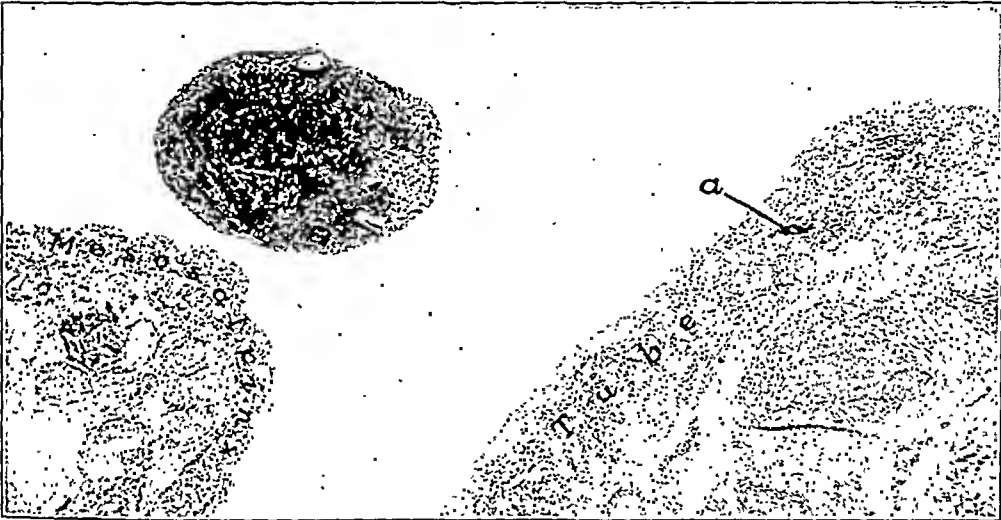
FIG. 131. A mature pedunculated polypoid implant attached to the mesosalpinx, from the same patient as the preceding ones. The structure of this implant is similar to the one shown in Figure 128. On the other hand there is no evidence of the extension of the growth into the preexisting tissues of its host nor of carcinoma in the lymph vessels of the mesosalpinx beneath it. $\times 25$.

FIG. 132. The small end of a sessile pear-shaped polypoid implant, near its base. This implant resembles in shape the smaller implant shown in Figure 122 and is attached to the right tube near the origin of the mesosalpinx. The structure of this portion of this implant is the same as that of the base of the implant proper shown in the preceding microphotograph. A section through the large end of this pear-shaped implant appears in Figure 144. Carcinoma is present in a subserosal lymph vessel of the tube at "a." What is the relation between the carcinoma in the implant and that in the subserosal tubal lymph vessel? $\times 25$.

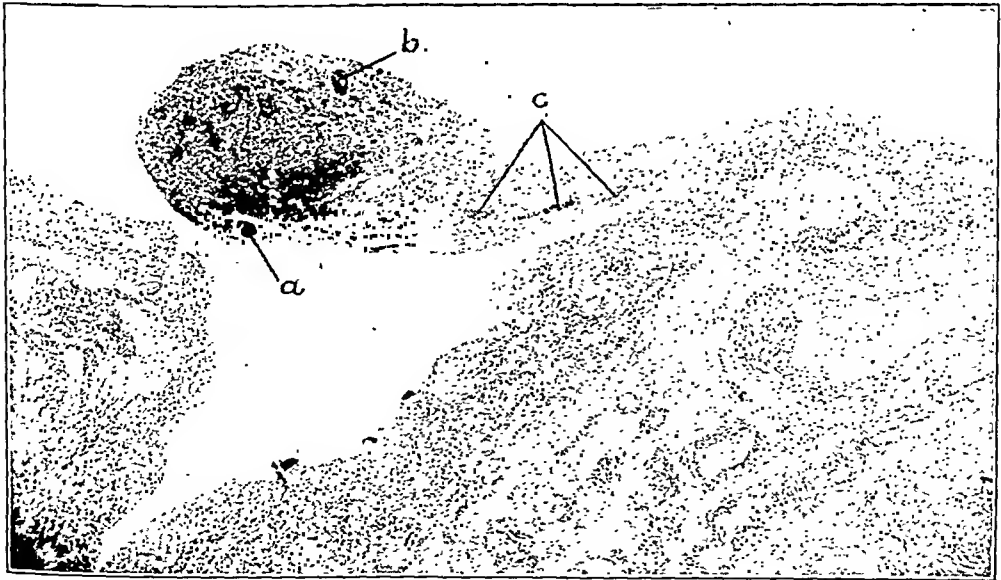
FIG. 133. The attachment of the sessile pear-shaped implant shown in the preceding microphotograph to the serosa of the tube. All of the tissue shown in this field except the carcinoma is preexisting. A horizontal section of a ridge of the tubal serosa to which the implant is attached presents under low magnification the appearance of the long slender pedicle of a polypoid implant. The carcinoma in this field is continuous with that shown in the preceding microphotograph. Carcinoma ("a," "b" and "c") is shown in lymph vessels. It can be demonstrated that the carcinoma in the first two vessels is continuous with the growth between them and that carcinoma "c" is a continuation of carcinoma "a." Note carcinoma is not present in the lymphatics of the mesosalpinx beneath the attachment of the implant shown in Figure 131 and that the growth in the implant has not invaded the mesosalpinx. See also similar implants on an epiploic appendage (Fig. 122) without carcinoma in the lymph vessels of the appendage. The conditions just demonstrated in the present implant are analogous to those shown in implants on the epiploic appendages of Figures 124 to 130 inclusive. I believe that the carcinoma in the lymph vessels of the tube, as in those of the last mentioned epiploic appendage, primarily came from the nearby implant and not from the ovarian tumor by retrograde lymphatic metastasis or permeation. $\times 25$.



131



132



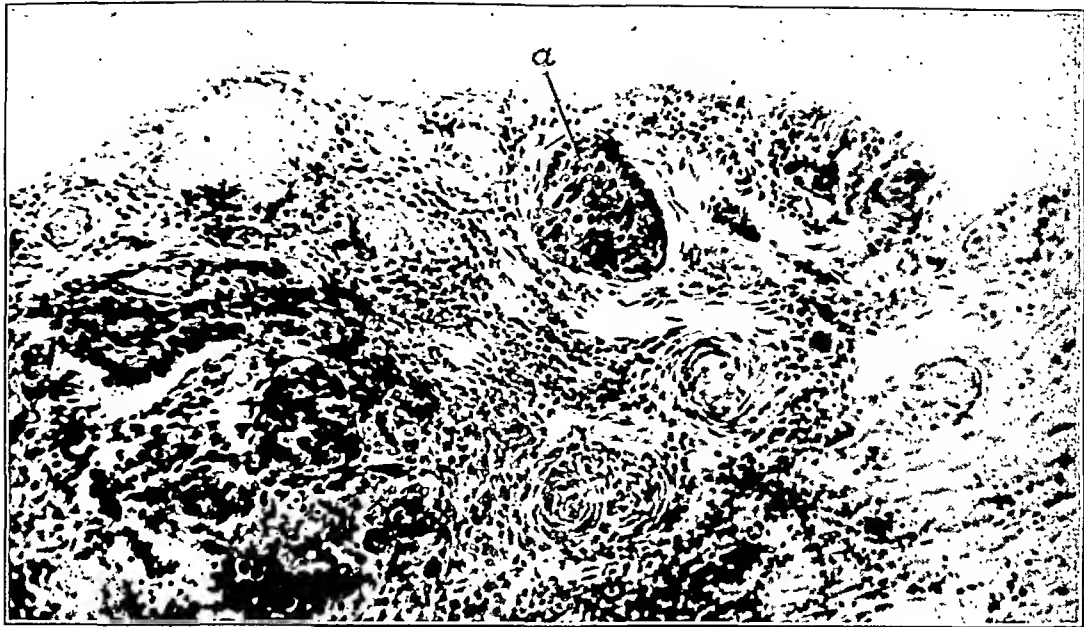
133

PLATE 113

FIG. 134. Higher magnification of a portion of the serosa and subserosa of the tube shown in the preceding microphotograph, from the same section. The tissues of the serosa, in the left-hand portion of this field, have been invaded by carcinoma from the implant which is attached to the tube in this situation. A lymph vessel is distended with carcinoma ("a"). The latter can be shown in the series of sections to be a continuation of the nearby carcinoma below and at its left. $\times 130$.

FIG. 135. Higher magnification of a portion of the pedicle-like structure shown in Figure 133. This structure is not newly formed but is a ridge of the tubal serosa which has been cut horizontally. Carcinoma in a lymph vessel is spreading either from or toward the implant. I believe the former. $\times 130$.

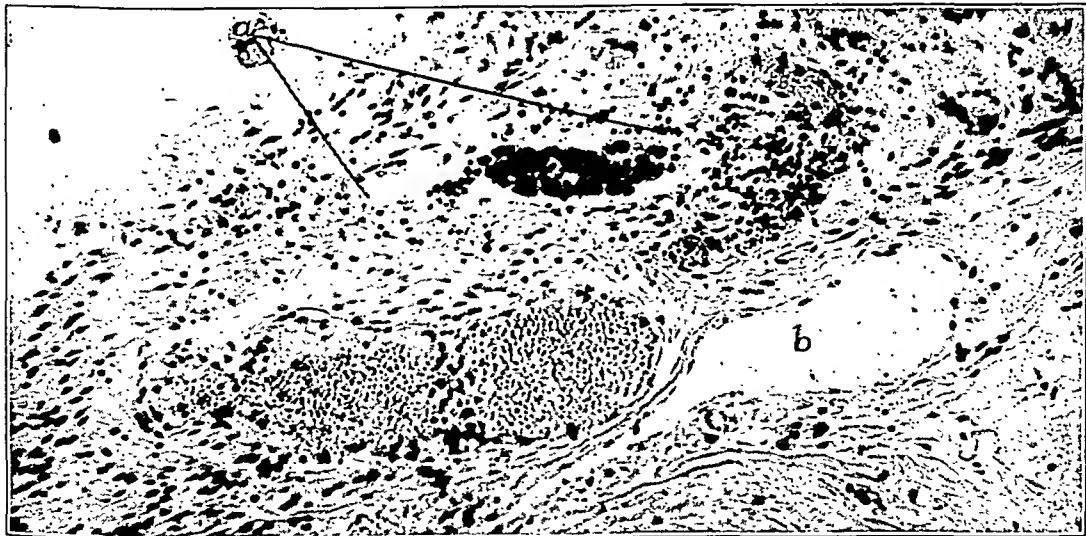
FIG. 136. Higher magnification of the tubal serosa and subserosa in which the carcinoma in a lymph vessel, indicated by "a" of Figure 132, is situated. Another lymph vessel or a portion of the one above is marked "b." The primary source of the carcinoma in lymph vessel "a," whether from the invasion of the ovarian lymph vessel by the ovarian tumor and an upstream metastasis from this source, or from a downstream spread of carcinoma implanted on the serosa of the tube with subsequent invasion of the underlying lymph vessels, is suggested in the two preceding microphotographs. $\times 130$.



134



135



136

FIG. 137. Longitudinal section of the distal end of the right tube including a portion of the mesosalpinx and the ovarian fimbriae along its free margin (Case 11). Carcinoma appears in this section in four situations. The most important one is a metastasis to a mucosal fold of the fimbriae marked by the arrow. The other three consist of various sized collections of cancer cells ("a," "b" and "c") occurring singly and in clumps. The cancer cells are held together by blood-stained debris which includes unidentified cells. These collections of cancer cells and the material about them are identical with those floating about in the ascitic fluid of this patient (see Fig. 140). They resemble rafts in the section. In this section a small raft ("a") appears just outside the abdominal ostium of the tube. A larger one ("b") is situated in the ampulla and still another but smaller one in the ampulla is marked "c." In other sections of this series they appear in all portions of the ampulla of the tube. In other sections a large raft can be seen in the lumen of the abdominal ostium, half of it lying between the fimbrial folds and the other half within the ampulla. Evidently these cells came from the abdominal cavity and have migrated into the lumen of the tube through its abdominal ostium. $\times 5$.

FIG. 138. A higher magnification of the metastasis to the mucosal fold of the fimbriae marked by the arrow in the preceding microphotograph. A polypoid growth of newly formed tissue, with carcinoma embedded in it, is attached to a mucosal fold by a short slender pedicle ("a"). The histological structure of this polypoid growth is similar to that of many pedunculated, polypoid serosal implants (compare with Fig. 131 from the same patient). An outgrowth of very early granulation tissue ("b") with cancer cells embedded in various portions of it (see Fig. 142) has developed on the under surface of the polypoid outgrowth. This is one way that implants on both the serosa and the mucosa may increase in size (see Figs. 101 and 107). Carcinoma is present, to the right, in the mucosal fold from which the newly formed stroma of the judged implant arose and is apparently continuous with that in the newly formed tissue (see also the next microphotograph). Carcinoma was not found in any of the lymph vessels of the mesosalpinx or wall of the tube in this situation. However, it is present in lymph vessels of this mucosal fold (see "c") and in those of a few nearby folds. I realize that this metastasis may possibly have arisen from the ovarian tumor by an upstream spread through the lymph vessels. However, I believe that the implantation of cancer cells in granulation tissue arising on the fimbrial fold with subsequent invasion of the fold and a downstream spread of the tumor in the lymph vessels of the mucosa is a more likely explanation of the condition just described (see Figs. 129, 130 and 133 from the same patient). $\times 25$.

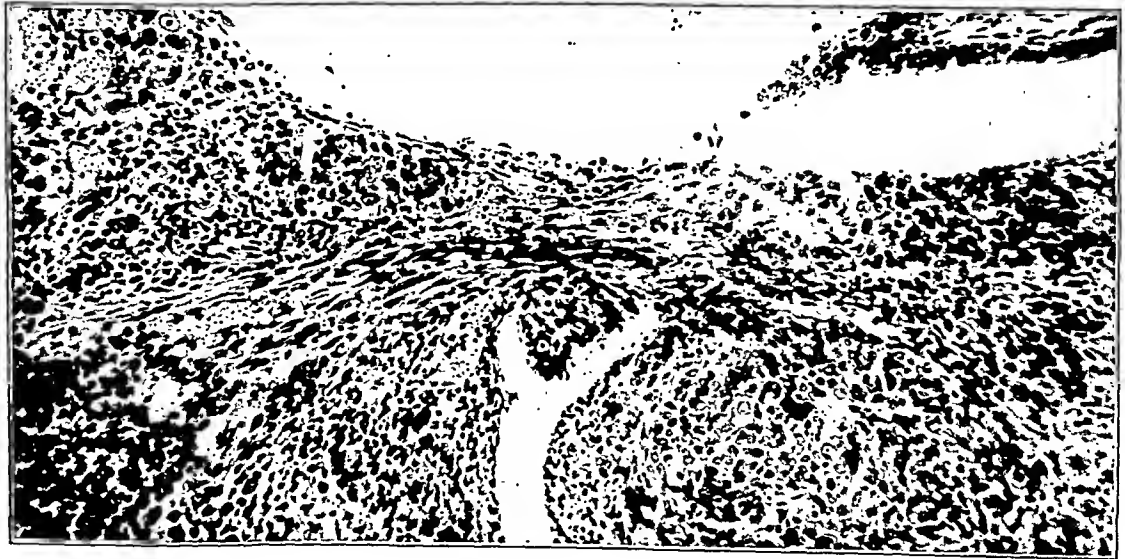
FIG. 139. Higher magnification of a portion of both the implant shown in the preceding microphotograph and the mucosal fold to which it is attached. The implant appears at the left and the mucosal fold at the right with the pedicle or bridge between the two. The continuity of the carcinoma in the two situations is very strongly suggested but cannot be positively demonstrated. Note that the stroma of the pedicle appears to be constricted. $\times 130$.



137



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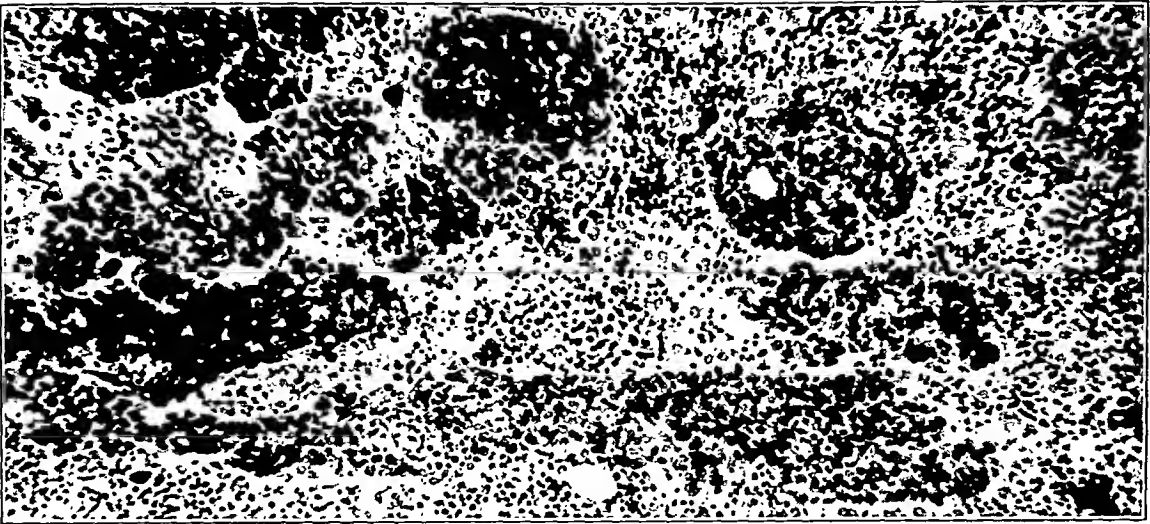
PLATE 115

FIG. 140. Section of the sediment obtained by centrifugalizing some of the ascitic fluid from Case 11. Cancer cells singly and in clumps are present in this sediment. While some of the cancer cells appear to be dead, others stain well and appear to be alive and possibly to have increased in numbers. The fimbriae of the tube shown in Figure 137 were immersed in the ascitic fluid. $\times 130$.

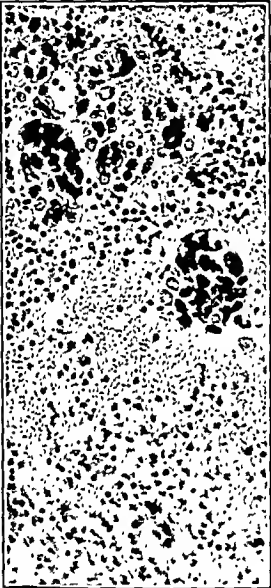
FIG. 141. Clumps of healthy appearing cancer cells in the collection of cells marked "a" between the fimbrial folds shown in Figure 137. Cells like these in contact with the surface of the mucosal folds might become implanted on them when a suitable soil is created for this phenomenon (see the next microphotograph). $\times 130$.

FIG. 142. Higher magnification of a portion of the mass of early granulation tissue arising from the under surface of the judged polypoid implant on the mucosal fold shown in Figure 138. Cancer cells similar to those shown in the preceding microphotograph are embedded in this tissue. The larger mass of cancer cells marked "a" is not completely surrounded by this tissue at its right. The small clump of cancer cells ("b") is embedded in the tissue as are also those (a little out of focus) marked "c." The pedunculated implant shown in Figure 138 is increasing in size by the development of granulation tissue, on its surface, in which cancer cells are becoming embedded. In like manner this implant may have arisen. Note that the cancer cells in the lymph vessel ("d") of the mucosal fold, have grown into a mass similar to the clumps of cancer cells in the ascitic fluid shown in Figure 140. The carcinoma in the mucosal fold shown in Figure 138 could easily have invaded the lymph vessels of that fold. $\times 130$.

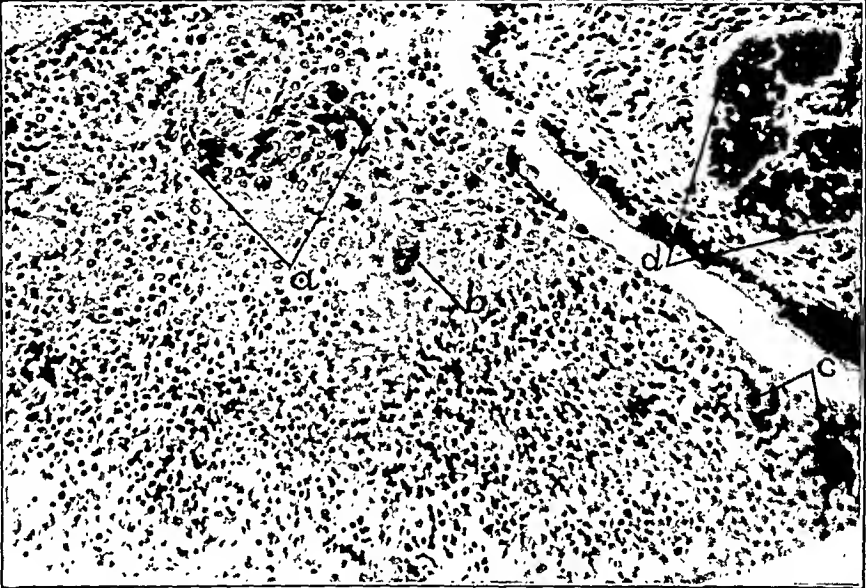
FIG. 143. Higher magnification of a portion of the smaller collection of cancer cells in the lumen of the ampulla of the tube indicated by "c" in Figure 137. I believe that these cells migrated from the abdominal cavity into the tube through its abdominal ostium. Compare with Figures 140 and 141 and note that the cancer cells appear to be alive. It is evident that the mucosa of the ampulla is exposed to these cells in the same manner as the peritoneum and the fimbrial mucosa. If a suitable soil should arise in the ampullar mucosa we would expect that these cells might become implanted on this mucosa as I believe they become implanted on the serosa and also on the fimbrial mucosa. $\times 130$.



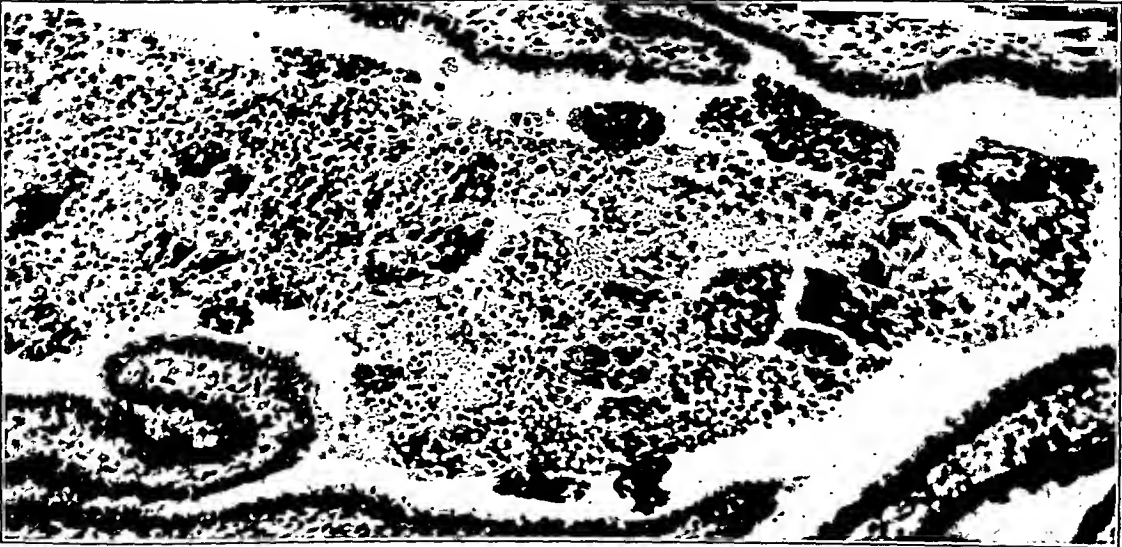
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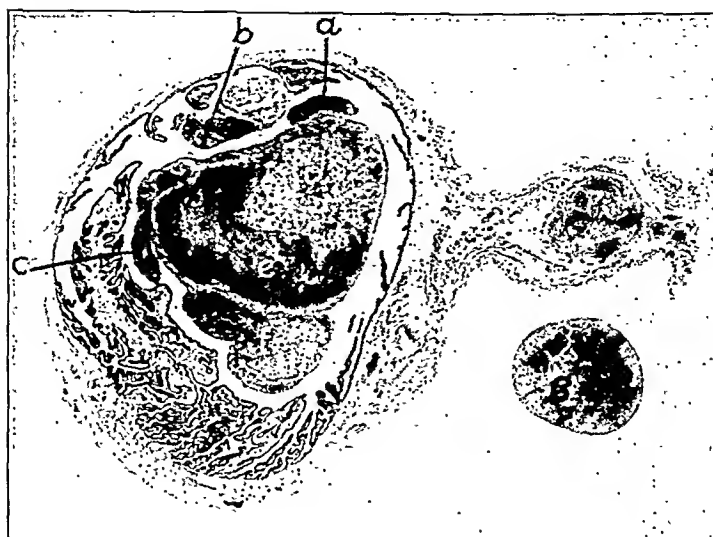


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FIG. 144. Cross section of the mid-portion of the ampulla of the tube shown in Figure 137. A serosal implant ("g"), similar to other serosal implants from the same patient (see Figs. 122, 123, 124 and 131), appears below the mesosalpinx. This implant is pear-shaped and is attached by its small end to the tube near the mesosalpinx (see Fig. 133). The tube is distended by an irregularly shaped conglomerate tumor. In the lumen of the tube above this tumor appear collections of cancer cells ("a" and "b") similar to those shown in Figures 137 and 143. A collection of cancer cells ("c") similar to "a" and "b" is partially fused with the tumor and is an indication not only of the way the tumor is now increasing in size, but also of the way the tumor may have grown. The nucleus ("d") of the tumor appears in the upper right-hand portion. This nucleus consists of a partially encapsulated rounded mass of vascular, newly formed connective tissue with carcinoma embedded in it and also of clumps of cancer cells, in various stages of implantation, on portions of its surface and thus exposed to the lumen of the tube. The nucleus is partially necrotic in its upper right-hand portion. This necrosis involves both the stroma and the carcinoma in it. The histological structure of the nucleus is similar to that of the serosal pedunculated polypoid implants (Figs. 122, 124, 128 and 131) and of the one pictured in this microphotograph, and also of the implant on the mucosa of the fimbriae shown in Figure 138, all from the same patient and all of judged implantation origin. The stroma of the nucleus differs from that of the implants just mentioned in that it contains more blood vessels and its connective tissue is more fibroblastic. Fused with the lower half of the nucleus is a large, deeply stained irregular shaped mass ("e") which for the most part is necrotic. The shadows of clumps of cancer cells in various stages of dissolution can be detected in the stroma of this mass. The mass just described and the nucleus have a common capsule with scattered clumps of viable appearing cancer cells embedded in portions of the capsule covering mass "e." Fused with the lower surface of this capsule is a pear-shaped mass ("f") smaller and paler than the mass ("e") above it. It is not as necrotic as the former and consists mainly of cells indistinguishable from the non-malignant undiagnosed cells about the cancer cells in the ascitic fluid shown in Figure 140, and about the collections of cancer cells in other portions of the ampulla shown in Figure 143. A few clumps of recognizable cancer cells and more and larger clumps of judged necrotic cancer cells are present in this mass. Fibroblasts, connective tissue and blood vessels cannot be detected in masses "e" and "f." It is clearly evident, from the study of this and other sections, that this conglomerate tumor has grown from the implantation of clumps of cancer cells, singly and collectively, on the surface of a nucleus of newly formed tissue which has the structure of a polypoid implant. It is also evident that the blood supply of this nucleus is inadequate, not only for the maintenance of the more recent attempted implantations but for the life of the nucleus itself. Hence the necrosis in these situations. $\times 5$.

FIG. 145. Higher magnification of a portion of the tumor, with a cross section of its pedicle ("a") and the ampullar mucosa beneath the pedicle, shown in the preceding microphotograph. This field is from a section at some distance in the series from the preceding one. In this section the tumor appeared much smaller than in Figure 144. There is an attempted implantation of clumps of cancer cells on its under surface. $\times 54$.

FIG. 146. A field similar to the preceding one and quite near it in the series of sections. The origin of pedicle "a" from a mucosal fold is shown. Blood vessels do not appear in this section of the pedicle but are evident in a nearby section which, on the other hand, does not show the origin of the pedicle or the carcinoma "b" and "c" in the mucosal lymph vessels as well as this section does (series of sections not quite complete). A further discussion of the conditions shown in these last two microphotographs appears in the report of this case (Case 11). $\times 54$.



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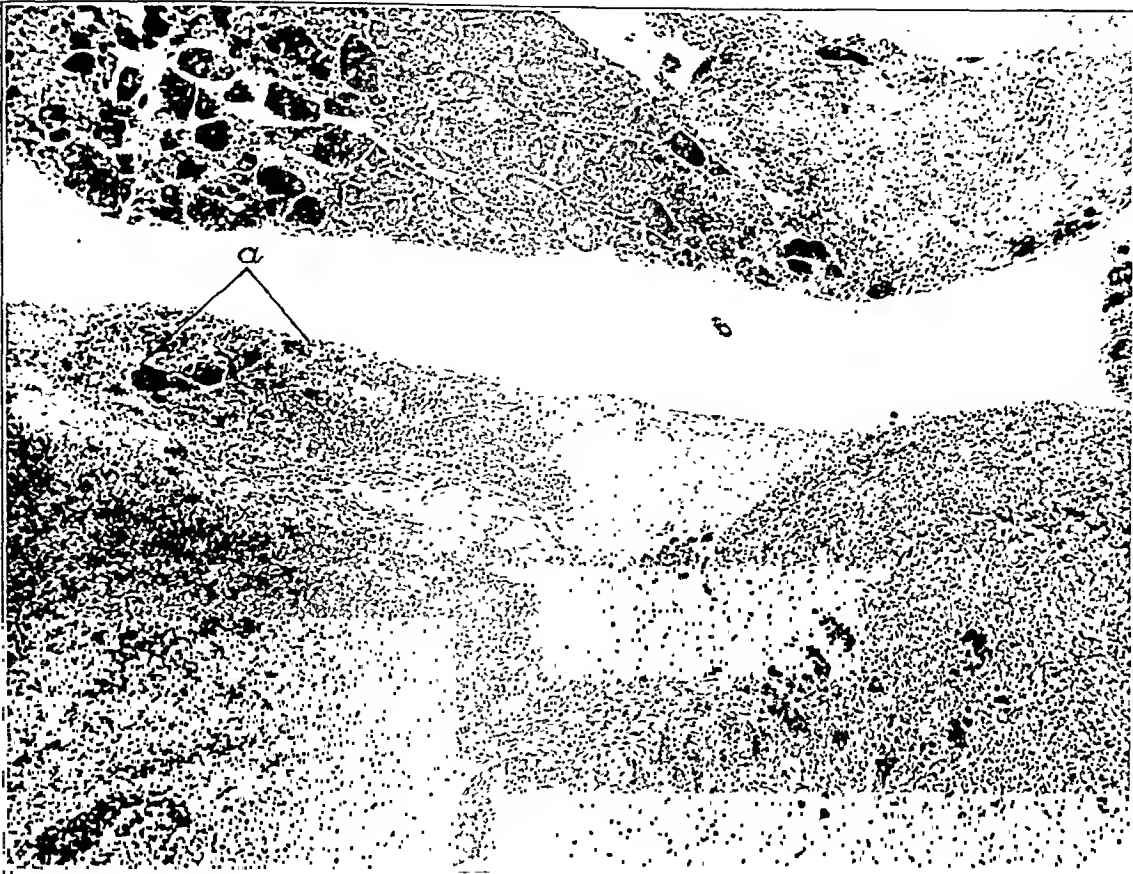
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FIG. 147. Higher magnification of a portion of the mucosal tumor (implant) and of the large collection ("b") of clumps of cancer cells above it (shown in Fig. 144). I believe that the clumps of cancer cells migrated from the peritoneal cavity through the abdominal ostium of the tube (see Fig. 137). The well organized vascular nucleus of the tumor is shown at the right with viable appearing carcinoma in it and on its surface. A portion of the necrotic mass "e" of Figure 144, which, I believe, represents the unsuccessful implantation of a large collection of clumps of cancer cells, similar to the one shown above the tumor, appears at the left. On the surface of the capsule of this necrotic portion of the tumor is an attempted implantation of cancer cells ("a"). $\times 54$.

FIG. 148. A clump of cancer cells in contact with the intact epithelium of the ampullar mucosa of the tube shown in the preceding section. There are many such clumps of various sizes in all portions of the lumen of this tube. Since the cells stain as well as the tubal epithelium it would seem that they might live a long time in the lumen of the tube as similar cells live in ascitic fluid and in the lumens of lymph vessels. These clumps of cells vary greatly in size and therefore may multiply in this situation even though mitotic figures are not evident. The latter were not evident in the primary ovarian tumor, in the growth in the lymph vessels or in the cancer cells in the ascitic fluid. Note the deeply staining cell in the normal appearing tubal epithelium beneath the clump of cancer cells. I wish I knew its significance. There is no appreciable local reaction on the part of the subepithelial tissues of the mucosal fold. $\times 130$.

FIG. 149. A field similar to the preceding one and from the same section. A small clump ("a") of detached cancer cells appears in the lumen of the tube. Above and to the right of this is a larger clump of cancer cells which is attached to the surface of the mucosa. There is a marked reaction, indicated by lymphocytes, in the subepithelial tissue of the mucosa beneath the attachment of the cancer cells. Lymphocytes have not only grown out between the mucosal epithelial cells but have actually replaced some of the latter. They also have extended into the clump of cancer cells. The phenomenon just described, the only one of its kind encountered in this series of sections, may well represent an early stage in one type of implantation of cancer cells on tubal mucosa. $\times 130$.

FIG. 150. Carcinoma of the mucosa of the ampulla of the tube from the same series of sections as the ones shown in the preceding microphotographs. Only one other tumor like this one was encountered in this series. I do not believe that they are primary carcinomas of the tubal mucosa. They more likely arose from the implantation and subsequent growth of cancer cells which had been floating about in the lumen of the tube (there are many such cells in this tube). The tumor shown here well may represent a later stage of the process shown in the preceding microphotograph. The reaction of the mucosal tissues beneath the attachment of the carcinoma is similar to that shown in the preceding microphotograph. Note that lymph vessel "a" is in close proximity to the growth. If the patient had not been operated upon at this time, but later, the carcinoma, in the meantime, might have invaded the nearby lymph vessel, and its pathogenesis might then have been attributed to metastasis from the ovarian growth through the lymph vessels rather than to implantation. $\times 130$.



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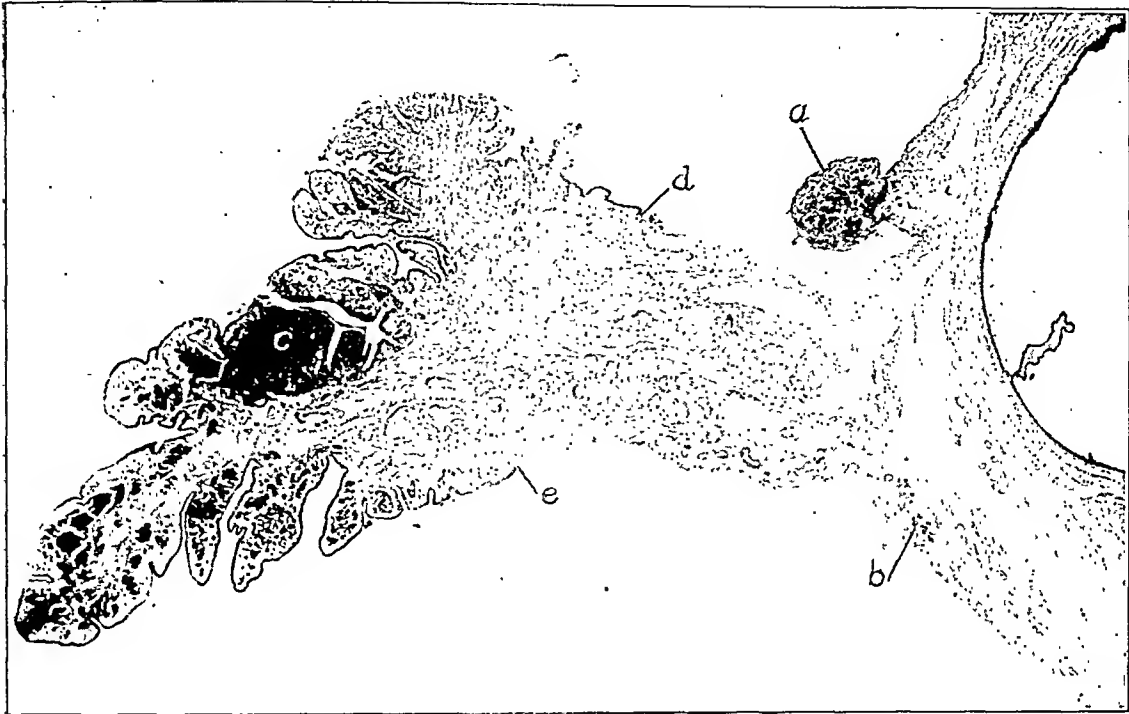
149



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FIG. 151. The free margin of the right mesosalpinx near the tube, including the fimbriae and a portion of the ampulla (hydrosalpinx), from a patient (Case 12) with a large papillary adenocarcinoma of the right ovary (judged secondary carcinoma of the left ovary and tube) and peritoneal carcinomatosis. A portion of the dilated tube appears at the right with a polypoid implant ("a") attached to its serosa. Cancer cells are present in a space ("b") lined by mesothelium. The lymph vessels of the fimbrial mucosa are filled with carcinoma as with an injection mass. In two situations, however, the carcinoma has extended beyond these vessels and invaded the tissues of the folds. Carcinoma cannot be detected in the deeper lymph vessels of the mesosalpinx proper but is shown in lymph vessels below the mucosal fold ("c") which is infiltrated by the growth. Two mucoserosal junctions are indicated by "d" and "e." Note that there is no indication of an anastomosis between the mucosal and serosal lymph vessels in these situations. The portal of entry of the carcinoma into the mucosal lymph vessels of the fimbriae was not detected, owing to an incomplete study of this specimen. The histological picture of the fimbrial folds is one resulting from lymphatic permeation, probably in this instance from the ovarian carcinoma. Implants were not detected in these fimbriae (compare with the fimbriae of the opposite tube shown in Fig. 154). $\times 10$.

FIG. 152. Cross section of the left ovary including a portion of the broad ligament at the right (Case 12). The right lower portion of this ovary has been invaded by carcinoma which well may have come from a growth arising on the surface of the ovary. This phenomenon is more evident in sections (though badly torn) from another block. A large papillary carcinoma appears on the upper left-hand surface of the ovary with encapsulated carcinoma below it. A pedunculated polypoid implant is marked "a." Two sessile polypoid implants ("b" and "c") appear on the serosa of the broad ligament. Portions of two sessile polypoid implants on the under surface of the ovary are marked "d" and "e." The encapsulated carcinoma "f" also well may be of implantation origin. The carcinoma to the left of this presents the appearance of having sunk into the ovary from its surface: the ovarian tissue has not completely encapsulated it (see arrow). The picture shown here is similar to that sometimes found in early ovarian endometriosis. I believe that the carcinoma of this ovary arose from the implantation of cancer cells on its surface, which had escaped from the large carcinoma of the opposite ovary into the peritoneal cavity. Carcinoma is present in a lymph vessel or vessels of the broad ligament, marked "g." Carcinoma was not found in the lymphatics of the broad ligament in the sections of the other block. I believe that the carcinoma in the lymph vessels, just described, probably came from the nearby ovarian tumor rather than primarily from the growth in the opposite ovary by a roundabout, retrograde lymphatic metastasis. $\times 5$.



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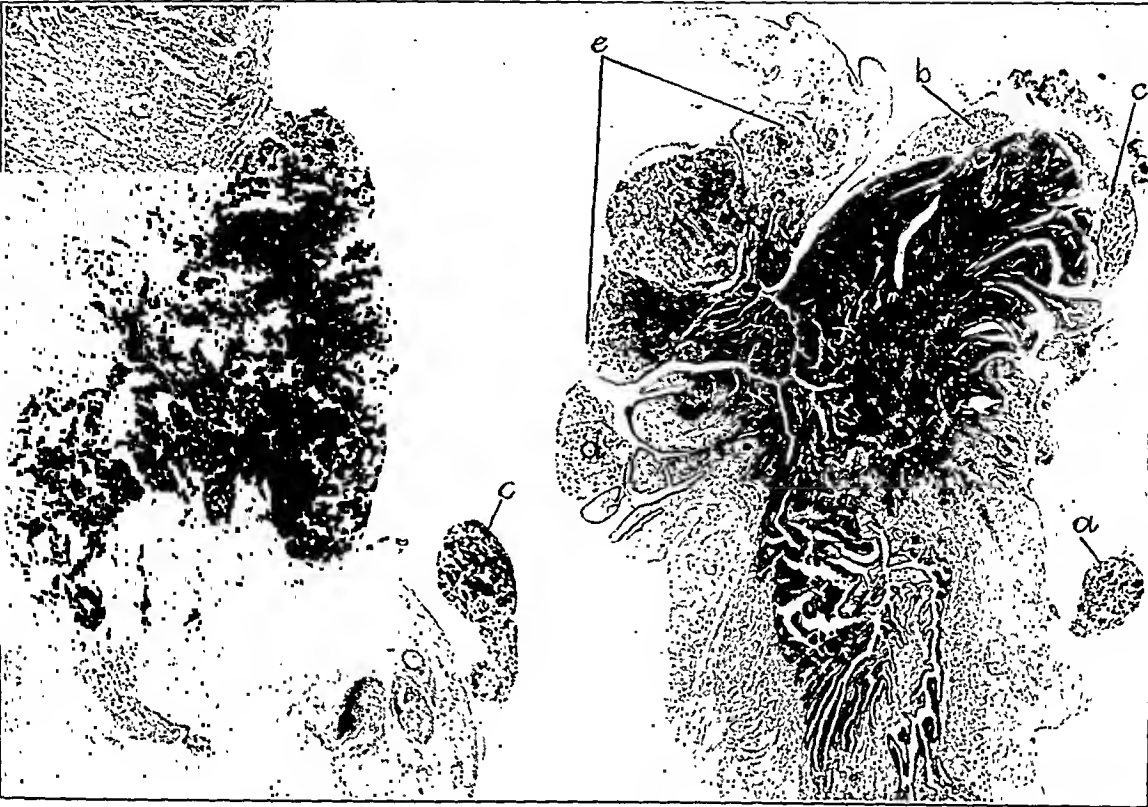
152

FIG. 153. A large sessile polypoid serosal implant in the bottom of the anterior cul-de-sac at the junction of the anterior uterine wall with the vesico-uterine reflection of peritoneum (Case 12). The carcinoma in this implant has invaded both the uterine wall and the reflection of peritoneum. Carcinoma is present in lymph vessels of both the uterus and the peritoneum with positive proof, in the latter situation, that it is continuous with the carcinoma in the implant. A pedunculated polypoid implant ("c") similar to "a" of the preceding microphotograph is shown, to the right, on the vesico-uterine reflection of peritoneum. The entire picture, shown here, is one resulting from the implantation of cancer cells on the peritoneum with subsequent invasion of their host and extension into the lymph vessels. $\times 10$.

FIG. 154. Longitudinal section of the distal end of the left tube including its fimbriae (Case 12). The carcinoma of the greater portion of the fimbrial mucosa is too far advanced to ascertain either the initial site of the secondary carcinoma of this mucosa or its pathogenesis. Many of the mucosal folds about the ostium of the tube are either partially or completely replaced by the tumor. The growth has spread over the surface of the fimbrial mucosa by replacing its epithelium and also has invaded its lymph vessels. The carcinoma has spread into the ampulla by replacing its mucosal epithelium like a primary tubal carcinoma. The growth has invaded the wall of tube about its ostium, at the right, penetrated the lymph vessels and, here, presents a picture similar to the invasion of the carcinoma in the preceding microphotograph. A pedunculated polypoid serosal implant similar to the one shown in Figure 153 is marked "a." Recent newly formed tissues which have arisen from the fimbrial mucosa, with cancer cells partially and completely embedded in them, are marked "b" and "c." A polypoid implant attached by its slender pedicle to a mucosal fold, similar to serosal implant "a," is marked "d." The area marked "e" presents two phenomena: one, apparently newly formed tissue with cancer cells growing in it, and the other, the invasion of the stroma of the mucosa including its lymph vessels by carcinoma. In many ways this area resembles the sessile polypoid serosal implant shown in the preceding section. We have demonstrated that carcinoma recently has become implanted in newly formed tissue arising on fimbrial mucosa of this tube. This suggests, but does not prove, that the initial secondary carcinoma in this situation also may be of implantation origin with a life history similar to that shown in the preceding illustration. $\times 8$.

FIG. 155. Higher magnification of a portion of the uterine wall beneath the sessile implant shown in Figure 153. Carcinoma in small lymph vessels is indicated by the arms of pointed "a." I believe that the space containing carcinoma, in the lower portion of the microphotograph, is a large sub-serosal lymph vessel. $\times 54$.

FIG. 156. Higher magnification of a portion of the carcinoma about the ostium of the tube shown in Figure 154. It represents an older portion of the secondary carcinoma but not necessarily its initial site. It presents the histological picture of a primary tubal carcinoma. $\times 54$.



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Sampson

Implantation Carcinoma of Tubal Mucosa

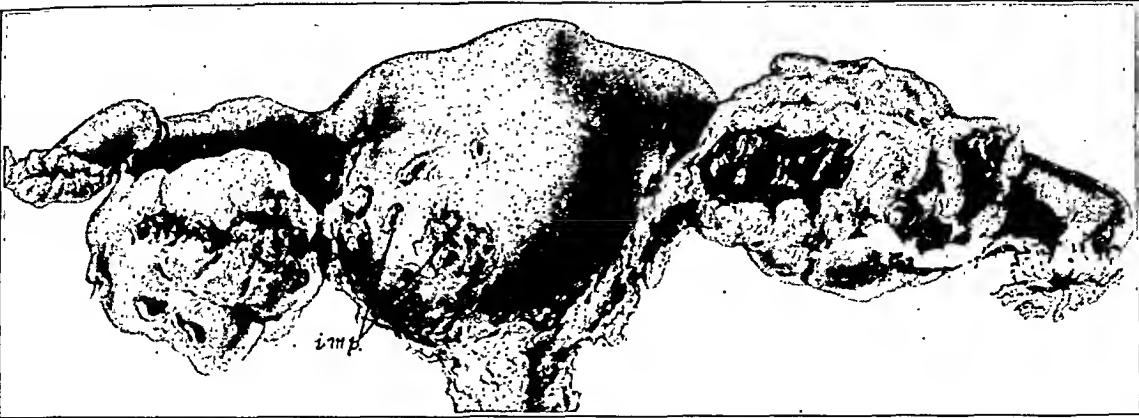
PLATE 120

FIG. 157. Posterior view of the uterus, tubes and ovaries from a patient (Case 13) with carcinoma of both ovaries and peritoneal carcinomatosis. The fimbriae of the right tube appear normal. Those of the left tube are thickened and retracted. The carcinoma of the right ovary is more advanced than that of the left, best seen in sections. The uterus contains multiple small myomas and a large mucosal polyp. Implants are indicated on the posterior surface of the uterus and also were present on many other pelvic structures. Natural size.

FIG. 158. An implant on the posterior surface of the uterus shown in the preceding illustration. Apparently carcinoma in the implant has invaded the uterine wall beneath it and reached some of the lymph vessels in this situation. For earlier stages in the life history of implants, similar to this one and from the same patient, see Figure 23 of previous paper⁹ and also the next microphotograph. $\times 25$.

FIG. 159. An early implantation of carcinoma on the mesoappendix, from the same patient as the preceding implant. Carcinoma does not invade the mesoappendix in this situation and cannot be detected in the lymph vessels beneath the implant. $\times 25$.

FIG. 160. An implant on the surface of the appendix near the attachment of the mesoappendix, at the left, from the same section shown in the preceding microphotograph. This implant is an old one: a portion of the original tumor has become necrotic and sloughed away causing the ulcer shown here. The carcinoma in the implant has invaded the wall of the appendix and has also extended between the appendix and the mesoappendix, at the attachment of the latter to the appendix. Carcinoma is present in the lymph vessels of the appendix beneath the implant and is judged to have come from the growth implanted on its surface rather than primarily from the ovarian tumor by a roundabout and retrograde lymphatic metastasis. For earlier stages in the life history of implants, similar to this one and from the same patient, see Figure 24 of previous paper⁹ and also the two preceding microphotographs. $\times 25$.



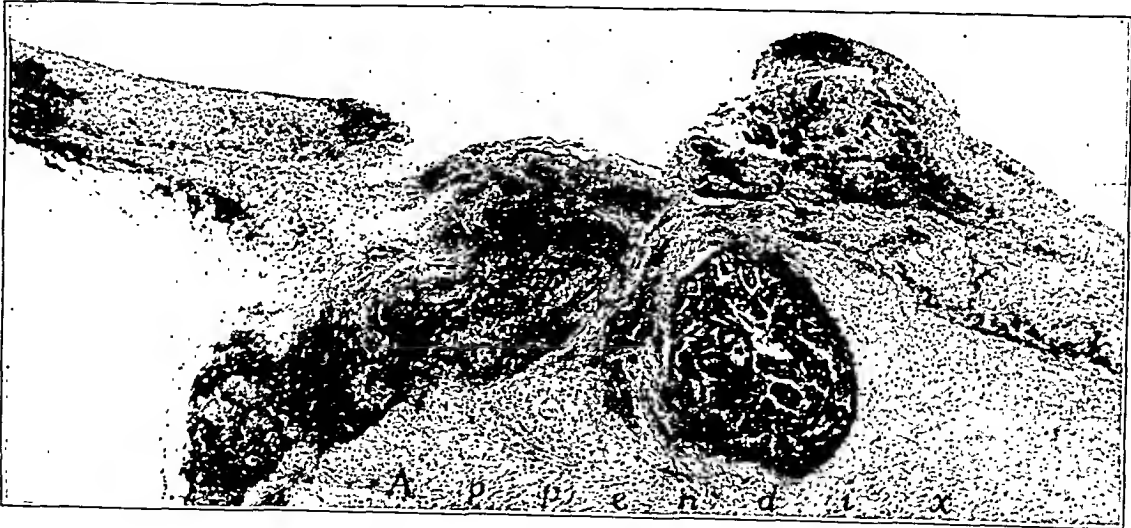
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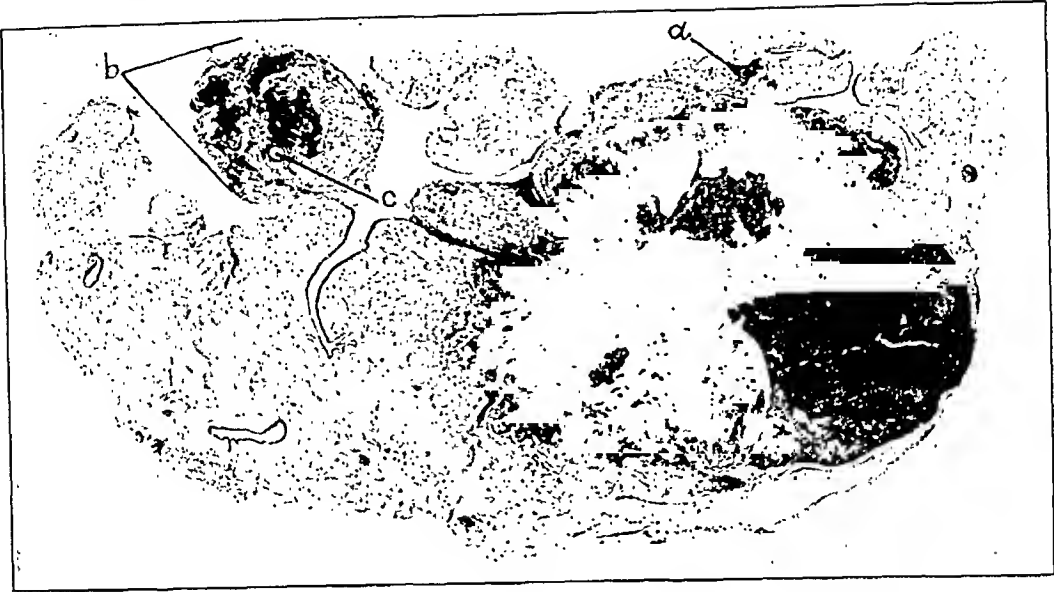
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PLATE 121

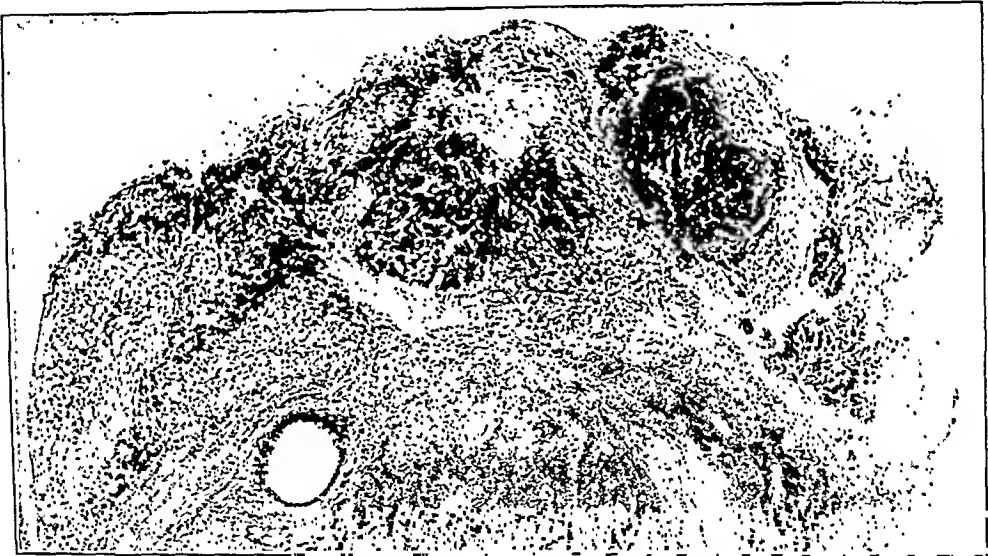
FIG. 161. Cross section of the fimbriated portion of the left tube shown in Figure 157. This section is through the largest portion of the secondary tumor and therefore judged to be possibly the oldest. The growth is so far advanced that it is impossible to determine either the initial site of its origin or its pathogenesis. A continuous extension from the primary ovarian tumor was excluded. Therefore it must be metastatic. The carcinoma here has invaded and destroyed many of the mucosal folds. It can be seen in the lymph vessel ("a") of one mucosal fold. An implant on the surface of a thickened mucosal fold is marked "b." In the center of this fold is a small amount of carcinoma. An inclusion of tubal epithelium is marked "c." $\times 10$.

FIG. 162. Higher magnification of a portion of the mucosal fold shown in the preceding illustration with an implant, marked "b," on its surface. Newly formed tissue has arisen on this fold showing both a very early stage in the implantation of cancer cells on its surface, at the extreme right, and also later stages in the encapsulation and growth of these cells in the rest of this tissue. The histological picture of this field is similar to that of carcinoma implanted on the serosa (see Figs. 158 and 159). Other sections in this incomplete series suggest that the carcinoma found in the deeper tissues of this fold may be continuous with carcinoma on its surface. Since carcinoma implanted on the serosa may invade the underlying tissues of its host, including the lymph vessels (see Figs. 153, 158 and 160), carcinoma implanted on the fimbrial mucosa should do the same. $\times 54$.

FIG. 163. Cross section of the tube pictured in Figure 161 and from the same series of sections. If the condition shown in Figure 161 indicates the oldest portion of this secondary tumor, that shown here should represent the further extension of the growth in the mucosal folds of the ampulla. Carcinoma is present in lymph vessels "a" and "b." The carcinoma in lymph vessel "a" can be demonstrated in other sections to be continuous with the growth in the mucosa above it. The condition just shown represents the results either of the implantation of cancer cells on the fimbrial mucosa with subsequent extension of the growth and a downstream invasion of the lymph vessels or an upstream metastasis through the lymph vessels from the ovarian carcinoma. The mucosal fold with an implant on its surface, marked "b" in Figure 161, also is present in this section. Compare the conditions shown in the last three microphotographs with those pictured in Figures 158, 159 and 160. Since cancer cells become implanted on the tubal mucosa in the same way (see Fig. 162) that they become implanted on the serosa and since the metastases shown in these six microphotographs have so much in common, it is possible that their pathogenesis and life history might be the same. $\times 10$.



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THE BEHAVIOR OF MURINE AND HUMAN LEPROSY IN FOREIGN HOSTS *

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Efforts to transmit either human or murine leprosy to other species of mammals result frequently in the development of local lesions that ordinarily heal without establishing a progressive disease. The injection of material from such lesions into a second series of animals usually, though not invariably, fails to produce any significant reaction. The inoculum for the first passage often contains lepra bacilli in enormous numbers and even though the infectious material is sterilized in the autoclave, acid-fast bacilli, quite normal in appearance, may be demonstrated for a period of many weeks after injection.¹ The suggestion is commonly offered that the lesion developing at the site of inoculation involves no multiplication of the bacilli but resembles the reaction that occurs in tissues in the presence of a foreign body. However, there is considerable support for the less conservative interpretation that lepra bacilli may multiply in a foreign host and produce an abortive infection without the usual progressive disease accompanied by extensive metastases. Neither human leprosy nor the analogous disease murine leprosy have been propagated in continuous serial passage in animals of other species.

MURINE LEPROSY

The abortive lesions resulting from the inoculation of human lepromas into lower animals fall short of affording a satisfactory method for experimental study. However, there occurs in nature a form of leprosy in rats which was first described by Stefansky² in 1903. Opinions diverge widely concerning the exact relationship of *Bacillus leprae* and *B. leprae murium*; Walker and Sweeney³ are inclined to regard them as identical, whereas Muir⁴ concludes that they are specifically or even generically distinct. The causative microorganisms and the histopathology of human and murine

* Received for publication March 25, 1938.

leprosy are remarkably similar in their essential features. The attempts to infect volunteers by the inoculation of human leprous tissue have resulted usually in failure; murine leprosy is readily transmissible by ordinary routes of injection from rat to rat. Our efforts have been directed toward securing well established progressive infections of rat leprosy in supposedly refractory species, the ultimate purpose of these observations being to secure some guide for the study of human leprosy in lower animals.

Much of the recent work in this country on rat leprosy has been carried out with the strain that has been propagated in white rats for many years by Dr. E. L. Walker of the University of California. Within a few months after subcutaneous injection local and metastatic lesions rich in lepra bacilli develop with regularity, a result that is in contrast with the less certain behavior of some of the freshly isolated strains.⁵ We are grateful to Dr. Walker for his courtesy in supplying us on several occasions with rats showing well established lesions of leprosy and in excellent condition for immediate use. The infectious material was suspended in testicular extract in view of the enhancing effect of such extracts in various types of infection. In particular, Walker and Hoffman⁶ have recorded the early and extensive development of lesions in animals inoculated with *B. tuberculosis* suspended in testicular extract. A preliminary test was made to see whether a similar effect might occur in the case of murine leprosy. The testes of a guinea pig were ground with a little sand and suspended in 10 cc. of physiological saline solution. A portion of this was mixed with an equal volume of saline suspension of murine leprosy bacilli and injections of this mixture were made subcutaneously into 4 white rats. Some of the suspension of lepra bacilli was diluted with an equal volume of physiological saline and injected into 4 control rats. Observations of this group of animals at various intervals left an impression that the use of testicular extract enhanced the development of both the local and the metastatic lesions. The effect, however, was not especially striking and eventually physiological saline only was used in preparing suspensions of lepra bacilli.

EXPERIMENTAL OBSERVATIONS

Animals: In addition to white rats we have followed the behavior of rat leprosy in other rodents, namely mice, rabbits and

guinea pigs. Young adult white mice of mixed breeds were used as supplied by commercial dealers. Subsequently some tests were made of the susceptibility of a few inbred strains of mice. Monkeys (*Macacus rhesus*), were also used in order to compare their susceptibility to murine and to human leprosy.

Routes of Inoculation: Especial attention has been given to injections into the brain and also into the spleen. The meninges of the brain are capable of supplying the types of mesenchymal cells essential for the growth of lepra bacilli and the spleen is particularly rich in such cells. In patients who show involvement of the nerves, the Hansen bacillus grows in mesenchymal cells within the nerve sheath; it has been mentioned only very rarely indeed as occurring in the ganglion cells.⁷ In spontaneous cases of rat leprosy the nerves remain unaffected.

Subcutaneous Injection: A group of animals was injected subcutaneously in order to facilitate the interpretation of the effect of using special routes of inoculation with this particular strain of murine leprosy. Infectious material from a subcutaneous lesion in a rat was suspended in testicular extract and injected subcutaneously into 2 monkeys (*M. rhesus*), 3 rabbits and 4 guinea pigs. Local lesions developed at the site of inoculation but these regressed spontaneously in the course of a few months. Several groups of white mice were injected subcutaneously with essentially similar results; 2 exceptional cases will be described subsequently. Instead of the interpretation that these various species are altogether insusceptible to murine leprosy, it seems probable that they are markedly-refractory but not entirely resistant. The small subcutaneous lesions (1 to 2 mm.) in mice ordinarily contain numerous lepra bacilli of normal morphology and their virulence has been demonstrated on several occasions. In two instances a small amount of material from subcutaneous lesions was used successfully for the serial passage of murine leprosy in mice.

Intracerebral Injections: For the first test of intracerebral inoculation a rich suspension of murine lepra bacilli in testicular extract was injected into 26 adult white mice, average weight 20 gm., 2 monkeys (*M. rhesus*), and 8 young rats, average weight 60 gm. The rats received about 1/30 cc. each, the mice about 1/60 cc., and the monkeys 1/4 cc. each.

It was not feasible to record complete data concerning all of the

mice. During the succeeding months after inoculation some died of intercurrent disease and the brains and even the viscera were frequently eaten by the survivors. However, observations were obtained at representative periods, including both short and long intervals. One mouse died 24 days after injection and a few intracellular lepra bacilli were seen in smears from the brain. In 1, which died 56 days after injection, cells were seen that were well filled with lepra bacilli; another was killed 79 days after injection and sections of the brain showed well developed lesions of leprosy. At 88 days after injection 1 mouse developed complete paralysis of the hind legs and was killed; smears from the brain showed occasional clumps of lepra bacilli. Especial interest attaches to a mouse that lived for a longer period. One year and 23 days after injection this animal was in an altogether critical condition and was killed. The autopsy showed well marked lesions of leprosy in the brain and extensive metastases widely distributed throughout the body resulting in a massive infection. The conclusion was obvious that this animal was dying of leprosy.

The 2 monkeys remained in good condition for several weeks. By the 37th day after injection 1 of them had developed well marked paresis of the extremities and was found dead 4 days later. Sections of the brain showed definite evidence that leprosy bacilli had multiplied extensively within the mesenchymal cells in the meninges and around the blood vessels. The immediate cause of death might very well have been increased intracranial pressure. The 2nd monkey became progressively weaker and died 2 months after injection. The autopsy findings were similar to those in the 1st monkey but in addition there was a complicating bilateral bronchopneumonia.

Under ordinary conditions rat leprosy usually runs a course of a year or more in its duration. Of the 8 young rats injected intracerebrally, 6 died or were killed when almost *in extremis* at irregular intervals between the 2nd and 4th months after injection, the short course of the disease being attributed to excessive intracranial pressure. Two of the 8 rats lived for 9 months. The extent of metastases depended chiefly on the length of life after injection; in these 2 rats there was a well marked infection of the cord with extensive involvement of the spinal nerves.

For confirmation of the preceding results a second series of

animals was injected intracerebrally with murine leprosy from the subcutaneous lesion of a rat as follows: 22 mice with lepra bacilli suspended in saline solution; and 2 rabbits, 1 monkey and 4 guinea pigs with lepra bacilli suspended in testicular extract.

Eighteen of the mice died within $3\frac{1}{2}$ to $5\frac{1}{2}$ months after injection. The sections showed extensive lesions of leprosy in the brain with metastases commonly in the spleen and occasionally in the liver. None of this group survived for an exceptionally long period, the last mouse dying 6 months and 19 days after injection. One of the 2 rabbits injected with murine leprosy died a week later of an intercurrent infection. The other rabbit died with some slight paresis on the 24th day after injection, and sections of the brain showed histological evidence of the multiplication of lepra bacilli, the exact cause of the paresis being undetermined. One month after injection the monkey died of some unknown cause and the sections also showed early signs of leprosy. The 4 guinea pigs remained well or died of some intercurrent disease and in no case were any lepra bacilli found microscopically. The last of these guinea pigs was killed and autopsied $1\frac{1}{4}$ years after injection.

Subsequently another group of 6 mice was injected intracerebrally with murine leprosy and each mouse developed a well marked infection. Rabbits, however, have behaved with irregularity. One was sacrificed 4 months after intracerebral injection and smears from the brain showed a few scattered lepra bacilli but there was no evidence of active infection. Another rabbit developed marked paresis of the hind legs 24 days after an intracerebral injection and was chloroformed. A cold abscess had developed in the ventricle on the side of the brain that was injected and smears of the affected tissue showed many lepra cells. Preliminary attempts to obtain serial passages in rabbits have failed.

Injection of Mice in the Spleen: The spleen was exposed by laparotomy under general anesthesia and injected with about $1/16$ cc. of a suspension of lepra bacilli from an infected rat. Of 10 mice injected in this manner, 5 died a few hours after the operation. Of the 5 survivors, 1 died 19 days after injection; microscopically sections of the spleen showed several foci of lepra cells. Two mice were killed at later intervals (4 months, 25 days, and 5 months, 22 days) and a massive infection of leprosy was found in the spleen. The 2 remaining mice were allowed to live for

longer periods. In 1 of these animals the abdomen was enormously distended with fluid in the peritoneal cavity, the spleen was enlarged, very hard on palpation and adherent to the peritoneal wall. The skin over this area had broken down, exposing granulation tissue characteristic of leprosy. At this time, $8\frac{1}{2}$ months after injection, this animal was in a critical condition and was killed. At autopsy there was one feature of especial interest. The liver under its capsule and on section was studded with whitish nodular lesions of leprosy of almost pin-head size, reminiscent of the gross appearance, for example, of the liver of an animal dead of bubonic plague. An identical picture was found in the remaining mouse of this group, killed 9 $\frac{1}{6}$ months after injection.

The intrasplenic injections were repeated with murine leprosy taken from the local lesion in a dilute brown mouse 13 $\frac{1}{5}$ months after subcutaneous inoculation. A saline suspension of material from this lesion was injected, after laparotomy, into the spleens of 3 white mice. The first death in this group occurred at 1 month and 3 days, and the second death at 4 months and 23 days after injection. Both animals showed a well developed leproma in the spleen with extensive miliary lesions in the liver. In the 3rd mouse a firm tumor was found on palpation of the left side of the abdomen, and this was interpreted as an enlargement of the spleen. The condition of this animal was rather critical and it was killed 4 months and 24 days after injection. There was a definite tumor in the spleen, but the palpable tumor in the abdomen was found to be a leproma (0.75 cm. in diameter) which had developed at the site of the incision in the peritoneum. A similar but smaller tumor (2 to 3 mm. in diameter) was found some distance away in the uninjured wall. It is obvious that after the injection into the spleen there is abundant opportunity for leakage of inoculum from the site of the injection, thus soiling the peritoneum with lepra bacilli.

Inoculation into the spleen often leads to the development of localized nodules at the site of injection accompanied by a widespread infection of the liver which in some cases has been more extensive than in the spleen itself. Metastatic infection of the spleen results in uniformly distributed miliary lesions which show but little tendency to extend to the liver. When the spleen is traumatized by the sudden injection of a rather large inoculum,

lepra bacilli might easily find their way freely and promptly through the veins into the liver. The injection into the spleen would become in effect a simultaneous injection of the spleen and liver. Two mice were injected directly in the liver with a strain of murine leprosy which had been maintained in mice for 16 months. In both cases extensive hepatic lesions developed. Also, in 1 of the 2 mice the omentum was studded with lepromas, this animal having been killed while still in good condition at an interval of 6 months after its injection.

Intraperitoneal Injection: In exceptional instances it was noted that white rats escaped infection after the intraperitoneal injection of murine lepra bacilli and on one occasion we lost this strain of leprosy through the failure of a rat to develop any lesions after inoculation by this route. Injections by the intraperitoneal route were omitted in our earlier observations on the susceptibility of mice. However, upon injecting mice in the liver or in the spleen it has already been noted that there is usually some gross leakage of the inoculum into the peritoneal cavity and in some of these animals lepromas have developed in the parietal peritoneum and in the omentum. Accordingly, a group of 17 white mice of mixed breed was injected intraperitoneally with murine lepra bacilli taken from an infected rat. These animals were sacrificed 6 to 7 months after injection. Fifteen showed gross changes, notably miliary lesions of the spleen and of the liver. Frequently the omentum was studded with lepromas. In several instances the epididymis was the seat of an exceptionally massive infection. The lungs usually escaped any extensive involvement. Two mice showed no gross lesions, but a careful examination of smears from the viscera and from the peritoneal wall of each resulted in the finding of a few isolated acid-fast bacilli. Similar infections were reported recently in "German mice" by Watanabe⁸ after intraperitoneal injection with murine leprosy.

Miscellaneous Routes of Injection: The counterpart of the more or less pure forms of nerve involvement by the Hansen bacillus in man does not occur in animals infected with murine leprosy and this type of lesion failed to develop under the following experimental conditions. Two monkeys (*M. rhesus*), and 2 rabbits were injected under general anesthesia in the sciatic nerve with a rich suspension of murine lepra bacilli. The final examinations were

made 8 to 24 months later. In each instance the nerve was normal in its gross appearance and preparations from the site of injection showed no acid-fast bacilli. A 3rd monkey was injected in the great cistern with murine leprosy 8 months ago and at present this animal appears to be in good health.

In generalized infections with murine leprosy the kidney, except for the invasion of its capsule, ordinarily though not invariably escapes infection. Three rabbits were injected in the kidney by direct puncture through the abdominal wall. These animals remained well and were sacrificed at intervals of 3 to 6 months. In 2 of these a few isolated clumps of acid-fast bacilli were found in sections of the kidney but there was no indication that an infection had been established.

Serial Passage: Murine leprosy has been maintained continuously since 1934 by passage in white mice of mixed breed, eight transfers having been made at irregular intervals by various routes of inoculation during a period of 4 years. Subcutaneous injections were used for the second and third passages and produced only small local lesions which did not suggest that this strain during the course of more than a year had become adapted to the mouse in any striking manner. Inasmuch as the subcutaneous lesions afforded only a minimal amount of infectious material the subsequent passages were carried out by other routes of inoculation. The fourth passage was made by direct puncture through the abdominal wall into the spleen without incising the skin. The spleen was injected successfully by this technique in several instances, but in some cases this procedure resulted merely in an intraperitoneal injection. The details of these passages are given in the accompanying table.

Mice of Known Genetic Stock: The use of animals of a high degree of genetic uniformity reduces the number of variables that one encounters in using mixed breeds. Dr. C. C. Little, Director of the Jackson Memorial Laboratory of Bar Harbor, Maine, has supplied us with several strains of inbred mice designated as, "M" leaden, "C 57" black, "D" dilute brown and an inbred albino strain. Dr. L. T. Webster of the Rockefeller Institute kindly sent us a stock of the Swiss strain of white mice which is now in common use in the study of a variety of infections. Preliminary observations, extending over more than 3 years, indicate that some of

these strains, particularly the ones designated as "dilute brown" and "C 57" black, might be used with fair success for the routine propagation of murine leprosy by subcutaneous injection. Frequently, tumors that are readily visible and palpable (0.5 to 1 cm. in diameter) have developed at the site of inoculation under the skin, and extensive metastases, particularly to the liver and spleen, have led to a progressive disease. Infections running a similar course have been noted with considerable regularity in mice of the

TABLE I

Serial Passage of Rat Leprosy in White Mice of Mixed Breed

Number of passage	Tissue of infected mouse used for injection	Route of inoculation	Interval between passages
First	* —	Intracerebral	—
Second	Brain and lung	Subcutaneous	1 year, 23 days
Third	Subcutaneous lesion	Subcutaneous	8 months
Fourth	Subcutaneous lesion	Intrasplenic	2 months, 14 days
Fifth	Spleen	Intracerebral	2 months, 21 days
Sixth	Brain	Intracerebral	5 months
Seventh	Brain	Intracerebral	7 months
Eighth	Brain	Intraperitoneal	6 months

* Subcutaneous lesion of white rat.

Swiss strain. The extensive, infiltrating subcutaneous lesions commonly occurring in rats have not been seen at any time in mice. Murine leprosy, which had been carried through two passages by subcutaneous injection in dilute brown mice during the course of 1 year and 2 months, was then injected into a white rat and characteristic lesions developed. It was not until the 3rd year of our experience with white mice of mixed breeds that any extensive infections were observed after subcutaneous injection of murine leprosy. In 2 mice from a group of 18, the lesion under the skin was insignificant in size (1 to 2 mm.) but the metastases in the liver and spleen and the course of the disease were altogether similar to the extensive infections observed in some instances in mice of inbred strains. The infrequency of this result is in contrast with the uniformity with which severe infections developed after other routes of inoculation. Lepromatous material taken directly from an infected rat was used for the inoculation of this group of mice.

CONTROLS WITH CULTURES OF ACID-FAST ORGANISMS

The conservative interpretation in the literature on the transmission of human leprosy to lower animals is based on the persistence of intact lepra bacilli at the site of injection over long periods. Even in the absence of any multiplication, their presence in the tissues incites a reaction that may imitate a true infection. Several groups of mice were injected with strains of non-pathogenic acid-fast bacilli, using *B. smegmatis*, a culture from timothy hay, one from butter, and a stock culture of a chromogenic organism labelled merely *B. lepra*. These microorganisms presumably have little or no ability to multiply in tissues. Heavy suspensions were made in testicular extract and injected intracerebrally, using 8 mice for each culture. In the first few days after injection many of the animals showed signs of excessive intracranial pressure and about one-third of them died; some of the survivors were sacrificed at later intervals for histological study.

The efficacy of the intracerebral route of inoculation was tested with cultures of the tubercle bacillus, using the avirulent BCG and a moderately virulent H 37 strain; 5 mg. of the latter, weighed in the moist state, infected guinea pigs with regularity. A heavy suspension of the avirulent culture of the human tubercle bacillus was made, using 40 mg. of the moist growth to 2 cc. of an extract of normal guinea pig testis. This was injected intracerebrally into 4 guinea pigs and into 9 white mice; none of these animals developed any evidence of infection. A moderately turbid suspension of the H 37 strain with the addition of testicular extract was injected into 9 white mice intracerebrally and into 9 controls subcutaneously. The mice injected intracerebrally died or were killed at intervals of 6 days to 5 months and 18 days. Lesions occurred in the brain which presented many of the features of murine leprosy. Moreover, in 2 of these mice macroscopic tubercles developed in the lungs and in 2 of the remaining mice the sections showed the presence of pulmonary tuberculosis. In 1 mouse, groups of macrophages laden with acid-fast bacilli were found in the spleen. The control mice injected subcutaneously died or were killed within 1 to 6 months after injection. No lesions and no acid-fast bacilli were found either at the site of injection or in the viscera.

HUMAN LEPROSY

During the course of this work we have had an opportunity to study one patient with leprosy, the lesions being of the nodular type in a rather early stage. A cervical lymph gland was excised under local anesthesia and ground lightly with sand in physiological saline, with the addition of testicular extract. The resulting suspension was not especially rich in lepra bacilli. Fifteen mice were injected intracerebrally. Two monkeys (*M. rhesus*) were injected intracerebrally and intradermally in the eyebrow and also subcutaneously. The mice were sacrificed at intervals varying from 2 weeks to 1 1/6 years. Four were autopsied 2 to 3 months after injection and smears of the meninges showed scattered lepra bacilli, but the inoculation of such material into the brains of white mice produced no infection.

The results in the monkeys were more interesting. The local reactions developing at the site of injection into and under the skin were insignificant and the animals remained in excellent health. In 1 of the monkeys a unilateral enlargement of the femoral glands was noted 1 year and 5 months after injection and some of these glands were removed under local anesthesia. Smears of a minute lesion showed large numbers of lepra bacilli but a further search of these glands failed to provide any material at all suitable for the inoculation of animals. This monkey died 1 year and 6 1/3 months after injection and the autopsy revealed no cause of death. No lepra bacilli were found in smears of the meninges, the viscera or of the superficial glands.

The 2nd monkey behaved in a manner analogous to that of its companion. One year and 4 months after injection this monkey was in a critical condition and was chloroformed. No gross lesions were noted at autopsy. Lepra bacilli were found in the glands of one axilla in moderate numbers and also in smears of the pia mater. There was nothing at all comparable to the massive infection which was seen in monkeys and mice injected with murine leprosy. The axillary gland was ground with sand and suspended in saline. Injections were made intracerebrally into monkeys (Nos. 3 and 4) and into mice, and also subcutaneously into 2 guinea pigs for the exclusion of tuberculosis. The guinea pigs were subsequently

killed and no lesions were found at autopsy. No infections with leprosy have occurred in the mice.

The observations on the monkeys (Nos. 3 and 4) consisted chiefly of the examination of superficial lymph glands removed by biopsy under general anesthesia. In Monkey No. 3, 7 months after injection an increase in size of the right inguinal glands was noted. Some of these glands that showed a few good clumps of acid-fast bacilli were inoculated into 2 guinea pigs and both animals remained free of any sign of tuberculosis. This monkey was in a semimoribund condition 11 $\frac{3}{4}$ months after its inoculation and was chloroformed. No gross lesions were found at autopsy. Smears of the dura mater showed a few acid-fast bacilli but none were seen in smears and sections of the lymph glands.

Some small glands in the right axilla were removed from the remaining monkey (No. 4) 11 months after its inoculation. Smear preparations showed occasional acid-fast organisms entirely consistent in their morphology with the Hansen bacillus. After an additional interval of 2 $\frac{3}{4}$ months a mass of about 12 small glands of from 2 to 8 mm. in length was removed from the left inguinal region. One of these glands showed a few acid-fast organisms and small portions of it were implanted in the brain of 2 white rats. On the following day this monkey was chloroformed. Occasional acid-fast bacilli were seen in smears of the pia mater and portions of the pia were implanted in the brain of 6 white rats, but the acid-fast microorganisms were very sparse and it is by no means certain that each animal actually received lepra bacilli. These inoculated rats, 8 in all, remained in good health and have been sacrificed at intervals of 10 to 15 months. In 2 of these animals smears from portions of the meninges showed rare acid-fast bacilli and in a 3rd smears from the choroid plexus contained many clumps of acid-fast microorganisms which were evidently the Hansen bacillus.

According to these results monkeys have shown more susceptibility to murine than to human leprosy. This situation is not necessarily illogical for the same conclusion might conceivably apply to man. Volunteers have frequently remained well after inoculation with human lepromatous lesions. These failures in the experimental transmission of human leprosy to its natural host emphasize the difficulty of establishing a progressive disease in

foreign species. The ease with which murine leprosy can be successfully transmitted by inoculation stands in distinct contrast with the behavior of human leprosy. However, intensive study has in general revealed additional similarities rather than emphasizing the differences between these two diseases.

SUMMARY AND CONCLUSIONS

1. Inoculation of murine leprosy into the brain resulted in the development of progressive lesions in monkeys (*M. rhesus*), in rabbits, in white rats and in ordinary white mice, but not in guinea pigs.

2. White mice of mixed breeds were also readily infected by injection in the spleen, the liver or the peritoneal cavity, but subcutaneous injection usually resulted only in an abortive infection. Mice of some inbred strains frequently developed well marked local lesions on subcutaneous injection and subsequently extensive metastases occurred.

3. Murine leprosy has been maintained by serial passage in white mice of mixed breeds for 4 years. Various routes of inoculation have been used. Massive infections have developed, providing excellent material for experimental purposes.

4. The bacillus of rat leprosy produced infection in the murine rodents, white rats and white mice, according to the manner of a microorganism developing in a favorable host, but the circumstances for its multiplication were apparently less favorable in rabbits and monkeys.

5. Monkeys (*M. rhesus*) were kept under observation for more than 1 year after inoculation with human lepromatous material. Lepa bacilli were seen in small numbers in smears of inguinal glands. Inoculations were made to other monkeys and a similar low grade infection developed during a period of more than a year. Portions of the pia mater of one of these monkeys were implanted intracerebrally in white rats; 15 months later many acid-fast bacilli were found in the brain of 1 of these rats. Briefly, human lepra bacilli were maintained in animals for a period of $3\frac{3}{4}$ years but no progressive disease and no active lesions developed.

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HISTOLOGICAL AND CYTOLOGICAL STUDIES OF MURINE LEPROSY *

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Histological studies of localized lesions developing at the site of inoculation in the various species of animals employed have been invaluable in determining whether or not true infection, in the sense of a progressive multiplication of the organisms introduced, had taken place. Animals showing generalized infection furnished excellent material for the study of the origin and development of the cells (lepra cells) which act as hosts for the lepra bacilli in various organs and tissues. The study of early lesions is particularly important for this purpose.

In rats the infection tends, for a time, to remain localized at the site of inoculation, but in all instances distant organs were eventually involved. This metastatic involvement occurs in an irregular manner, but sooner or later, if the animal lives long enough, nearly every organ and tissue may be massively involved. As has been brought out above, massive infection by direct injection into an organ usually resulted in the rapid development of extensive lesions there. The kidney was comparatively resistant to infection perhaps because this organ contains fewer mesenchymal cells of the type that readily become infected. No histological or cytological differences were observed between the local and metastatic lesions produced in mice and those produced in rats. Certain peculiarities of the lesions developing in monkeys and in rabbits will be brought out later.

In general, the pathological changes are closely parallel to those in human leprosy. Lepra cells, the cytoplasm of which is densely packed and often distended with bacilli, increase at the expense of normal tissues which undergo extensive pressure atrophy. Cell nuclei, which are never invaded, are usually normal in appearance,

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but occasionally are compressed and pyknotic. They are often flattened along the periphery of the cell, giving a "signet ring" appearance like that of the normal fat cell. The vacuolated or foamy appearance of the cytoplasm of infected cells, which is characteristic of the human lesion, has only very rarely been observed in our material. Giant cells are often present in large numbers.

The observations made are in harmony with the belief that murine lepra bacilli are capable of multiplying only within cells. Extensive areas of cell degeneration within lepra nodules, resulting probably from infarction, are frequently seen. In such areas extracellular bacilli set free by the dissolution of their host cells persist in enormous numbers apparently because of their great resistance to autolysis and heterolysis. Such bacilli are often disseminated in tissues during the process of preparing the sections. In no instance has the distribution of organisms appeared to justify the assumption of extracellular multiplication.

Lepra bacilli are commonly believed to be capable of growing only in mesenchymal cells of a certain type. Various workers, including Oliver,¹ Henderson,² and Lowe,³ have concluded that the lepra cells are derived from the cells that constitute the reticuloendothelial system. This concept, with certain exceptions that will be noted below, is in general supported by the present studies. In taking up lepra bacilli the cells of the reticuloendothelial system are merely carrying out one of their normal functions, the phagocytosis of lipid structures that are physiologically useless or detrimental to the organism. It is the ability of the ingested bacilli to multiply within these cells, instead of being metabolized there, that makes possible the development of progressive and eventually fatal lesions. As the lesion progresses new lepra cells are formed, not only from the local mesenchymal cells, but also by mitotic division of preëxisting lepra cells, so that the process is in some respects analogous to a neoplasm. Lepra cells are found in mitosis with sufficient frequency to suggest that the organisms, or some product of their metabolism, stimulate cell division. The lepra cells do not, however, acquire any of the morphological characteristics of malignant tumor cells.

Mesenchymal Tissues: Cells that are commonly considered to be members of the reticuloendothelial system, such as the Kupffer

cells of the liver and the cells lining sinuses and forming the reticulum of spleen, lymph nodes and bone marrow, were found, by a study of the earliest lesions, to be initially infected. Isolated Kupffer cells, for example, were often infected (Fig. 3) and frequently distended with bacilli. Ordinary vascular endothelium, including that of arterioles, venules and precapillaries, was never found infected and was almost invariably in a flattened quiescent state, even when these vessels were within nodules composed almost entirely of lepra cells. Smooth, striated and cardiac muscle fibers likewise appeared invariably to escape infection, although often studied in locations where they must have been adequately exposed. The same statement may be made regarding cartilage cells and also serosal and meningeal lining cells. The observations regarding the latter two types of cell are particularly valid, because these cells were very frequently seen as single rows of flattened, uninfected cells overlying tissue composed almost entirely of lepra cells. Polymorphonuclear leukocytes, which were present in the lesions in relatively small numbers, often contained bacilli but never in such numbers as to suggest multiplication within these cells. Cells that could be recognized as lymphocytes, plasma cells and eosinophiles were always free from infection. It will be noted that the cells thus far described which were found infected are precisely those commonly considered as members of the reticulo-endothelial system on the basis of their reaction to intravenously injected finely divided foreign substances.

The question of the reaction of fibroblasts and fat cells to infection could not be answered definitely by these studies. Large areas of ordinary connective and adipose tissue often became replaced by tissue which appears to be composed entirely of lepra cells, with a network of thin walled blood vessels. It seems probable that both the fibroblast and the lipoblast may eventually become infected, but one cannot rule out the possibility that they may disappear by pressure atrophy at the expense of lepra cells originating from histiocytes and by mitotic division of preëxisting lepra cells. The mesenchymal cells immediately adjacent to the sarcolemma of striated muscle fibers, regarded by some workers as specialized cells responsible for the formation of sarcolemma, become infected very early (Fig. 6).

In early lesions involving the testis isolated groups of interstitial

cells were often found infected (Fig. 2). This observation is in harmony with the view that certain of these interstitial cells belong to the reticuloendothelial system. In late lesions the interstitial cells appeared to become uniformly infected; here again, perhaps, as a result of pressure atrophy of those cells that were not originally invaded. After intracerebral inoculation infection commonly spread in the subarachnoid tissue over the entire surface of the brain, reaching the interior of the brain by following along the walls of the blood vessels (Fig. 1). In several instances infection also spread in the subarachnoid tissue of the spinal cord and produced extensive lesions of the cauda equina and peripheral nerves. Atrophy of the nerves was evidently due to the conversion of the mesenchymal cells into lepra cells and not to actual invasion of the nerve fibers. Ganglion cells were not found infected.

In the kidney, where infection was infrequent and rarely progressed to a point where lesions were grossly visible, the cells initially infected were mesenchymal cells in glomeruli and in the walls of intertubular capillaries and larger vessels. In the adrenal isolated mesenchymal cells or small clusters of mesenchymal cells were frequently infected. Such cells were found in both cortex and medulla but were, in several instances, most numerous at the junction of cortex and medulla. In bone marrow both extravascular and intravascular mesenchymal cells appear to be initially infected. Eventually by the proliferation of such cells the marrow becomes entirely replaced by lepra cells, the marrow spaces become greatly enlarged, and there is marked atrophy and disappearance of bony trabeculi (Fig. 7).

Epithelium: In general, it may be said that epithelial cells of all types were relatively resistant to infection. Liver cells, pancreatic acinar and islet cells, gastro-intestinal epithelium, and mucous gland and bronchial epithelium were invariably found to be free from infection, even when embedded in nodular masses of heavily infected mesenchymal cells.

In rare instances ependymal cells and neuroglia cells appeared to be lightly but definitely infected. In 1 rat lepra bacilli were found within epithelial cells of the epidermis in such large numbers as to indicate that intracellular multiplication had taken place. This rat was inoculated intracerebrally and extensive metastases

developed in the cervical lymph nodes. Cells forming all three layers of the epidermis overlying these nodes were infected, and localized multiplication of these infected cells had obviously taken place, sometimes producing small raised nodules with hyperkeratinization and sometimes causing localized areas of downward growth into the corium. In 1 mouse, also, the epithelial cells lining the tubules of the testes (Fig. 2) and the ducts of the epididymis were extensively invaded, so that the infected structures could easily be selected with the low power lens. This mouse was injected in the spleen with a suspension of lepra bacilli and showed extensive secondary involvement of the inguinal nodes and testis.

These observations were so infrequent that they should be considered as exceptions rather than the rule. Their chief importance lies in their suggestion that the relative resistance of epithelial cells to infection depends on their feeble phagocytic powers or on the inability of the bacilli to enter them, rather than upon intracellular conditions that are inhibitory to the growth of lepra bacilli.

Marchoux and Sorel ⁴ in 1912 studied the skin lesions of murine leprosy and noted in certain areas the presence of lepra bacilli in epithelial cells, an observation that has not attracted the attention of subsequent workers.

LESIONS PRODUCED IN MONKEYS AND RABBITS

Although definite evidence of progressive local and metastatic infection of rat leprosy in both monkeys and rabbits was obtained there is reason to believe that *B. leprae murium* grows less readily within the cells of these species than in those of rats and mice. In general, infected cells contained fewer organisms and infected tissues, stained by the Ziehl-Neelsen method, were never definitely red in color when examined with the unaided eye, as they commonly were in rats and in mice. Although numerous miliary lepromas were found in the livers of monkeys after intracerebral inoculation, lepra bacilli were scarce in such lesions and were found only after prolonged research. The cells composing these lepromas also differed from the lepra cells seen in rats and mice in that they showed the cytoplasmic vacuolization which is characteristic of the human lepra cell.

LESIONS PRODUCED BY INTRACEREBRAL INJECTION OF OTHER ACID-FAST ORGANISMS

Mice surviving the intracerebral injection of non-pathogenic acid-fast organisms never showed metastatic lesions. The organisms introduced called out many macrophages and neutrophiles. Organisms were found in large extracellular masses, but they were also taken up in large numbers by both macrophages and neutrophiles. A few days after injection these organisms showed evidence of degeneration, and after about 3 weeks they had entirely disappeared, although the inflammatory reaction was undiminished in intensity. No evidence of multiplication of these non-pathogenic organisms in the tissues was obtained, and the lesions produced by them could be readily distinguished from those of leprosy.

Avirulent tubercle bacilli (BCG) persisted longer in the brain, and several weeks after injection were found in large numbers in macrophages, presenting a picture more closely resembling that of leprosy. Organisms never distended the cells greatly, however. It was not possible to determine whether or not multiplication of these organisms had occurred in the tissues, but metastatic lesions did not develop.

With the virulent strain of the tubercle bacillus (H 37) lesions were produced in mice which simulated even more closely those of leprosy and which were accompanied by extensive metastatic lesions in the lungs (Fig. 8) and spleen. In these metastatic lesions organisms were present in enormous numbers and were largely within macrophages. These tuberculous lesions were not caseous and were often practically identical with those of leprosy, although the tubercle bacilli themselves could be identified because of their larger size and morphological characteristics. These metastatic tuberculous lesions developed only after intracerebral inoculation; subcutaneous inoculation of similar doses did not result in localized or metastatic infection.

SUMMARY

Lepra cells in murine leprosy are derived largely from mesenchymal cells belonging to the reticuloendothelial system. Exceptionally, however, epithelial cells, specifically those of the epidermis, testicular tubules and epididymis, became distended with

lepra bacilli. This observation suggests that the relative resistance of epithelial cells to infection may depend on the inability of lepra bacilli to enter them, rather than on intracellular conditions unfavorable to their growth.

In infected rats and mice surviving for a long time the tissues of practically all organs were extensively replaced by non-vacuolated lepra cells distended with bacilli, but kidney tissue contained relatively few of these cells.

In the progressive local and metastatic lesions produced with *B. leprae murium* in rabbits and monkeys lepra cells were often vacuolated and acid-fast bacilli were much less numerous in these lesions than in those produced in rats and mice, and were found only after prolonged search.

Non-pathogenic acid-fast bacilli, injected intracerebrally, were taken up by macrophages and neutrophils, but disappeared from the lesions in a few weeks, never producing metastatic lesions.

Virulent tubercle bacilli, although innocuous when injected subcutaneously into mice, produced progressive and metastatic infection when injected intracerebrally into these animals. The lesions were non-caseating and the tubercle bacilli were found largely within macrophages, so that these lesions closely resembled those of leprosy.

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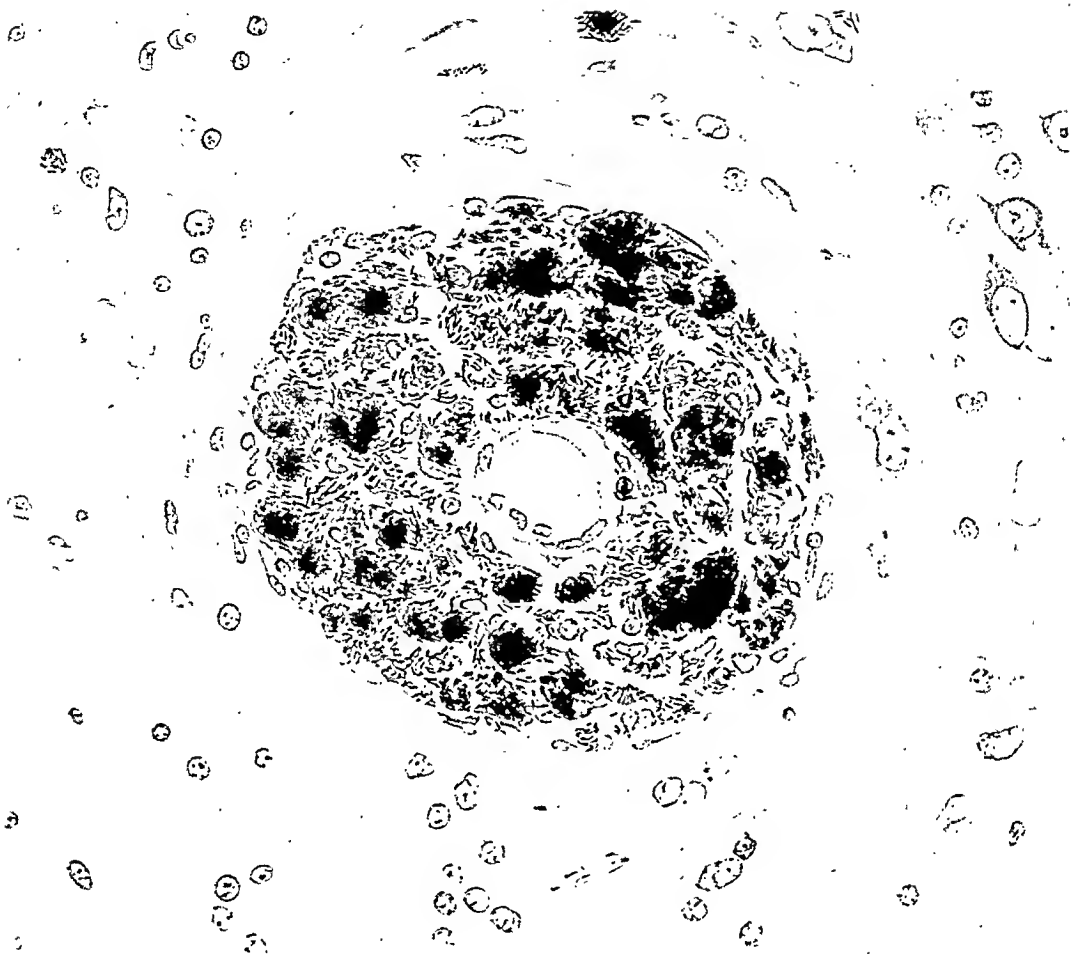
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DESCRIPTION OF PLATE

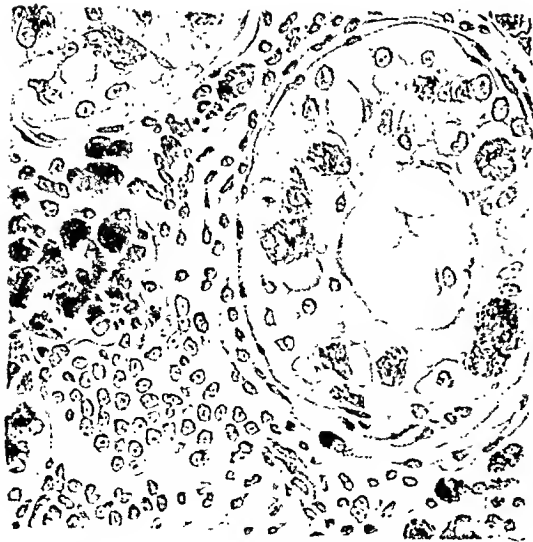
All sections were prepared from tissues fixed in 10 per cent formalin in 95 per cent alcohol and stained by the Ziehl-Neelsen method.

PLATE 122

- FIG. 1. Cerebral cortex of mouse after intracerebral injection of murine lepra bacilli showing phagocytic cells laden with acid-fast bacilli filling the perivascular space. The vascular endothelium is not infected. $\times 570$.
- FIG. 2. Mouse testis after intrasplenic injection of murine lepra bacilli. Note infection of tubular epithelial cells and patchy distribution of lepra cells in the interstitial tissue. $\times 400$.
- FIG. 3. Liver of mouse after intracerebral injection of murine lepra bacilli showing infection of isolated Kupffer cells. $\times 1300$.
- FIG. 4. Spinal nerve of rat after intracerebral injection of murine lepra bacilli. Lepra cells within the nerve bundle are derived from the mesenchymal cells there. The nerve fibers are not infected but disappear from pressure atrophy. $\times 570$.
- FIG. 5. Stomach of mouse after intracerebral injection of murine lepra bacilli showing lepra cells of mesenchymal origin. $\times 570$.
- FIG. 6. Striated muscle in rat leprosy showing infection of mesenchymal cells adjacent to the sarcolemma. $\times 570$.
- FIG. 7. Skull of rat after intracerebral injection of murine lepra bacilli showing accumulation of lepra cells in marrow space and consequent atrophy of bone trabeculae. $\times 570$.
- FIG. 8. Lung of mouse after intracerebral injection of virulent tubercle bacilli (H 37). The alveoli and alveolar walls contain many macrophages laden with tubercle bacilli, simulating the appearance of lepra cells. $\times 570$.



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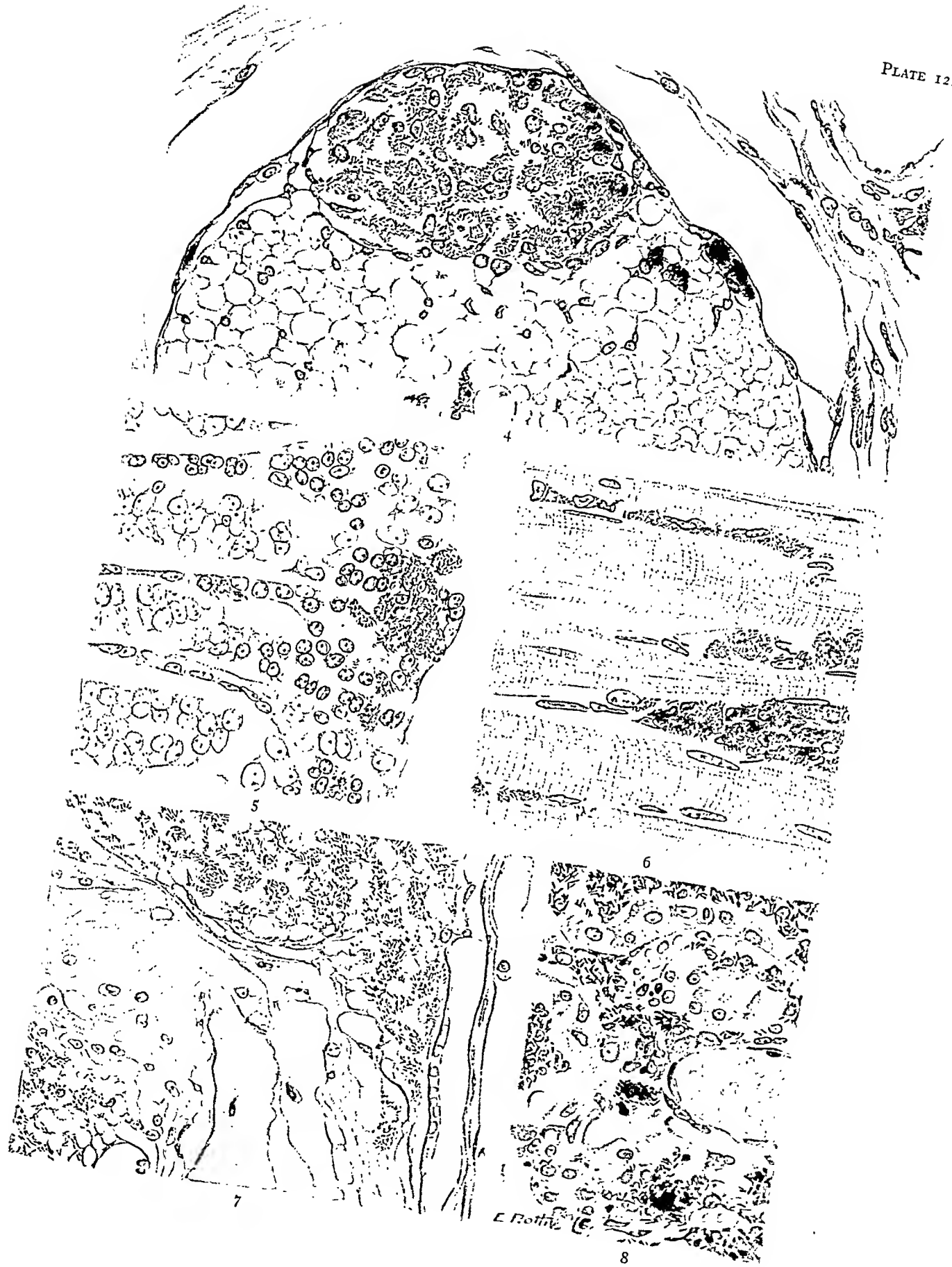


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E. Platt.

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E. Roth

MORPHOLOGICAL VARIATIONS OF TUMOR CELLS *

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Routine histological examination of a large group of malignant epithelial tumors during the past five years revealed in a number of instances distinct variations of individual cells or groups of cells in a single tumor. These variations were so extensive as to render the classification of these tumors difficult. In a series of cases it was not possible to classify these tumors, and diagnoses of "predominantly one type of tumor," such as "predominantly squamous cell carcinoma" had to be made. It was thought of interest to study in detail the histological appearance of such tumors, to see whether or not their complexity could be explained on the basis of collision or combination tumors and to see how such variations interfere with their gradations and affect the prognosis for the patient.

MATERIAL AND METHODS

This study is based on a selected group of various tumors derived from the routine material sent to the histological laboratory from the tumor clinic and the various departments of the Michael Reese Hospital. Those tumors were selected for study which in routine examination showed more than one apparent type of tumor cells. Fifty such tumors were studied. The majority were malignant tumors arising in the skin or mucous membranes, such as the oral cavity, larynx, esophagus, rectum and uterus. Blocks from these tumors were cut, and frozen and paraffin sections were made. In some instances serial sections were cut from individual blocks. Iron hematoxylin without counterstains and the hematoxylin-eosin stain were used, in addition to various special stains whenever deemed necessary.

RESULTS

Carcinoma of the Cervix and Uterus: There were 20 cases of carcinoma of the cervix and uterus where a definite diagnosis of

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carcinoma consisting of one type of cells could not be made. Most commonly this occurred in the type of carcinoma consisting mainly of transitional or spindle shaped cells in addition to other epithelial structures. These tumors arose either from the portio uteri or from the cervix and were apparently rather young, slightly ulcerated, but not well defined. Histologically there were fields of typical mature squamous cells with keratinization in addition to nests of squamous cells which were unevenly stained, showed atypical mitoses and were undoubtedly malignant. Other fields revealed accumulations of more cuboidal or spindle shaped cells resembling those seen in the basal cell layer of the epidermis; either between these cells or close by, thin spindle shaped nuclei with hardly any cytoplasm were recognizable, these cells resembling connective tissue cells. Transitional forms from the basal cell type of cells to the latter were often encountered. Other fields of the same tumor showed small, round or oval shaped cells with deeply stained nuclei. All these types of cells exhibited various degrees of anaplasia and atypical mitotic figures. The outstanding feature was the fact that the various types of cells were intermingled with each other so that squamous cell islands with or without keratinization were often surrounded by thin spindle shaped cells, or small anaplastic cells, and often transitional cells of the basal cell variety were found adjacent to the squamous cells. In individual sections it was sometimes possible to designate which of the tumor cells was predominant, but in the sections cut serially a diagnosis was barely possible.

In 3 instances sections of the tumors revealed large adenomatous structures which showed unquestionable evidence of malignancy. In addition to these structures there were more or less circumscribed areas consisting of squamous cells and transitional cells which in general varied in size, shape and staining quality and also contained atypical mitotic figures. These islets of squamous and transitional cells were present in many foci, which, as serial sections revealed, did not communicate with one another. They also were not particularly pronounced at the edges of the adenocarcinomatous structures.

Carcinoma of the Skin: There were 11 cases in this series where a diagnosis of carcinoma consisting of one type of cell could not be made. Clinically these were as a rule early tumors, as far as the

history was concerned. Most commonly the face was involved and the lesions on gross appearance and location were rather typical of the basal cell type of carcinoma. Histologically also there were fields characteristic of basal cell carcinoma. Nests of epithelial cells, polyhedral, slightly cuboidal and spindle shaped, and often arranged in elongated groups, invaded the subcutaneous tissue. These were distinctly separated from the overlying epidermis. The nuclei were large, vesicular with relatively large nucleoli, and the cytoplasm was scant. Here and there small empty cystic spaces were encountered lined by groups of tumor cells. The overlying epidermis, when not ulcerated, was often hypertrophic and hyperkeratinization was frequent. However, in the midst of the basal cells just described a number of horny pearls were noted and in other fields typical squamous cells or prickle cells with intercellular bridges were encountered adjacent to the basal cell nests. The squamous cells varied only slightly in size and mitotic figures were occasionally noted. Often these squamous cells were found in the midportions of the carcinoma, while the basal cell structures formed the peripheral portions of the tumor. Occasionally cystic structures, as described above, which were similar to those seen in the adenoides cystic variety of carcinoma, typical basal cells and squamous cells with keratinization were found in a single section. By serial sections it could be shown that the squamous cells were nowhere continuous with the squamous cell layer of the epidermis.

Melanotic Tumors: Although it is well known that melanotic tumors vary in different stages and that the structures of malignant and melanotic neoplasms and their metastases present wide variations, two tumors which to a large extent consisted of melanotic cells may be cited. One, obviously a malignant melanotic tumor found in the skin of the leg, measuring grossly 3 by 6 cm. in diameter, histologically showed fields consisting of large spindle shaped cells with large nuclei and dark brown intracellular and extracellular pigment granules. The nuclei were dark and oval. These cells resemble those seen in spindle cell sarcomas. In other fields the cells were large, square or polygonal, with large, lightly stained vesicular nuclei. These two cell types did not at all resemble each other. In the second tumor, which was benign, the tumor cells were rather small and the cytoplasm not well defined, the cells forming a sort of syncytium. Intracellular and extra-

cellular pigment granules were abundant. In addition, areas of squamous cells with or without keratinization were found in the midst of the tumor. Other fields revealed the typical basal cell types of tumor cells. Again, the various cell types were found in the midportion of the tumor and not confined to the peripheral regions. In short, the tumor consisted of nevus cells, squamous cells and basal cells.

Carcinoma of the Lip, Mouth, Epipharynx and Esophagus: There were 11 cases of carcinoma of the mouth and esophagus. Three of the latter were autopsy specimens. Histologically squamous cells were seen with varying amounts of keratinization, the latter much more rare in the esophageal carcinomas. The additional cell type here again was the basal cell variety, best described as spindle or oval cells similar to those seen in the mucosa of the urinary bladder (transitional cells). These cells were arranged in cords and rows invading the submucosa throughout. Many fields of the tumors of the esophagus consisted entirely of these types of cells. Others again showed these cells intermingled with malignant squamous cells. All the cells showed evidence of anaplasia and mitotic figures were abundant.

As far as the tumors of the epipharynx are concerned reference is made here to the tumors investigated by Salinger and Pearlman¹ which also came under my observation. In a number of instances three pathologists, of whom I was one, disagreed as to the type of tumor, whether it was a squamous cell carcinoma, transitional cell carcinoma or lymphoepithelioma. This apparently was due to the fact that various structures, each characteristic of one of these tumors, were present in the individual tumors. According to which of the various constituents was thought to be predominant, the various respective diagnoses were made.

Rectal Tumors: There were 6 cases in this series in which the histological examination also revealed apparently quite different structures. One was typically adenomatous, with many small or larger tubules, lined by one or two layers of cuboidal cells exhibiting atypical mitotic features and distinctly infiltrating the deeper layers. In addition there were epithelial cells arranged in clusters. These cells were polygonal or more or less round, also showing definite evidence of malignancy. These cells did not form a separate entity but were seen scattered throughout the adenomatous

structures. Occasionally areas of keratinization surrounded by tumor cells were seen.

DISCUSSION

Summarizing the findings it may be repeated that in a number of carefully studied tumors arising predominantly in the skin or mucous membranes, not a single type of tumor cells but two or more apparently different types of tumor cells were found. The incidence of various types of tumor cells in malignant conditions of the cervix uteri was noted by a number of investigators. As a matter of fact the grading of cervical carcinoma is based on the recognition of these various cell types. Chambers² speaks of "various types of squamous cell carcinoma" and in classifying them as "adult, spindle, keratinized, transitional," and so on, types, he describes all the types of tumors encountered. The principal question involved, however, is whether or not the anaplastic type and transitional type of carcinoma are squamous cell carcinoma or are primarily transitional carcinoma. There are many cases on record of simple squamous cell carcinoma, of anaplastic carcinoma without squamous cells, and of transitional cell carcinoma without squamous cell features. Also, in comparing cervical carcinomas with skin carcinomas, it seems evident that just as the basal cell carcinoma and the squamous cell carcinoma constitute definite entities, so the transitional type of tumor of the cervix, as of any other mucous membrane, is a special, clearly defined entity, apparently the corollary of the basal cell tumor arising in the epidermis. In other words it seems that the squamous cell carcinoma of the cervix is one type of tumor, and the transitional cell carcinoma is another. As the result of careful examination of malignant tumors because of the interest in the grading of tumors (Chambers²), and also as a result of this study, it must be emphasized that there are in addition to these two clear-cut tumors a number of tumors arising in the cervix which show the characteristics of both squamous and transitional cells.

An interesting group of carcinomas of the uterus are the so-called adenoacanthomas, 3 of which are included in this series. These tumors, apparently rare (Stein and Torek³), may arise in the portio uteri, cervical canal or in the endometrium. Because of the presence of malignant adenomatous structures and squamous

cells the term adenoacanthoma is used. It is apparently significant that the squamous cells are found more or less in the form of islands in the midst of the tumor. This would speak against the belief of metaplasia of cylindrical tumor cells into squamous cells, because such a metaplasia would rather involve one area instead of appearing in minute regions here and there throughout the adenomatous structures. It seems more likely that these tumors arise from regions which *a priori* reveal both glandular structures and islets of squamous cells, as described by Meyer.⁴

The skin tumors in this series consist mainly of squamous cells and basal cells. It seems that islands of squamous cells and keratinization are more frequently present in the basal cell carcinoma than *vice versa*. Ewing⁵ classifies epidermoid carcinoma as adult hornifying squamous carcinoma, transitional cell carcinoma and basal cell carcinoma, indicating three distinctly different types. However, he states that in rare cases of basal cell carcinoma the cells may assume pavement characteristics and spines; hornification and pearl formation may appear in traces. Geschickter and Koehler⁶ state that Grade IV squamous cell carcinoma resembles a basal cell carcinoma on close examination. They also state that Grade II carcinoma reveals undifferentiated cells which show distinct nuclei and binucleated forms in transition between basal and squamous cells. They described a mixed basal cell and squamous cell carcinoma occurring in a case of xeroderma pigmentosum and mention that in cutaneous basal cell lesions where metastases occur there usually will be found on careful microscopic study a definite transition to squamous cell epithelium. Such a tumor consisting of basal and squamous cells is referred to by Montgomery⁷ as mixed basal-squamous cell carcinoma. This author also stressed that the basal cell epithelioma is not a morphological entity, but may through metamorphosis become a basal squamous cell epithelioma or even a squamous cell epithelioma. It is interesting in this respect to note that Ewing⁵ states that squamous epithelium tends to hypertrophy when invaded by alien cells and that hyperkeratosis is common in such instances. Martin and Stewart⁸ described carcinomas of the skin which they term spindle cell epidermoid carcinoma because of the peculiar spindle shaped cells present. These tumors most often seemed to be a variety of scar tissue carcinoma. Similar tumors, which reveal histologically malignant squamous and

spindle shaped epithelial cells (transitional cells) are also described in mucous membranes of the lip. Because of the presence of the spindle shaped epithelial cells or transitional cells they are somewhat similar histologically to tumors arising from the esophagus, larynx, lungs, cervix, bladder and urethra. It may be pointed out especially that these authors further state that these tumors sometimes are erroneously referred to as carcinosarcomas. Gordon⁹ describes an apparently somewhat similar tumor which he called intra-epidermal epithelioma with epithelioma adenoides cysticum. As this study shows, it seems quite evident that not only in the unusual instance, but in a number of seemingly simple basal cell carcinomas, epithelial structures were encountered which undoubtedly are squamous in character.

There are melanotic tumors on record which histologically resemble carcinoma (melanocarcinoma) and those which resemble sarcomas (melanosarcoma). As pointed out before, it is just the melanotic tumor which seems to be characterized by the morphological variations of its constituents. The first case here reported clearly shows in individual sections both types, sarcoma-like and malignant epithelial-like cells. The primary lesion was a tumor on the lower extremities with early metastases in the groin. The other tumor belonging in this group revealed basal and squamous cell features and characteristic nevus cells. It is known that basal cell tumors may be confused clinically with melanomas. Because of the pigmentation in the former, Mallory¹⁰ thought that they might arise from hair follicles. Eller and Anderson¹¹ mention basal cell epitheliomas with excessive pigment formation. Goldsmith and Freudenthal¹² relate instances of epithelioma adenoides cysticum with basal cell type of cells, pearl formation and pigmentation. Affleck¹³ shows pictures of melanoma with squamous cell hyperplasia, and Becker¹⁴ speaks of melanotic epitheliomas, meaning hyperplasia of the surface epithelium with abnormal evolution of the majority of the malpighian cells which were often enormously hyperpigmented. He also described pigmentation in basal cell carcinomas. Ewing⁵ stated that with melanomas the swollen hyperchromic and pigmented epithelium is often difficult to distinguish from tumor cells. He also noted pigmentation in basal cell tumors.

The relevant tumors arising in the oral cavity probably fall in the classification of those named by Quick and Cutler¹⁵ "transi-

tional cell epidermoid carcinoma." These tumors were thought to arise either from transitional epithelium or from squamous epithelium which, in its growth, has lost its adult epithelial characteristics and has assumed anaplastic features. These authors stressed the fact that adult squamous characteristics, such as hornification, spines and pearl formation, are absent. In this series, however, the tumors showed in addition to the cells just described, which were termed transitional cells, foci of squamous cell features such as typical malignant squamous cells, keratinization, and so on.

Summarizing, it may be stated that in a group of various single tumors several distinct cell types were found. Ewing has drawn attention to variations of tumor cells in metastatic lesions and in recurrent tumors. He states that in metastases the original tumor structure might not be recognizable. Epidermoid carcinoma may appear as round or spindle cell growths; adenocarcinoma of the uterus may recur after curettage as epitheliomas, and melanoma may recur as round cell, diffuse and perivascular pigment-free tumors. In this study, however, it is shown that in some original tumors variations of cells of which the tumor consists may occur. The question arises whether these variations of cells constitute evidence of anaplasia of a single tumor and signify different ages of cell groups, whether they can be interpreted as evidence of two tumors growing into each other and forming one tumor, or whether (not necessarily indicating anaplasia) they may indicate what might be called morphological variations of tumor cells, as will be discussed later.

As to the possibility of the various cell groups signifying merely anaplasia, the following may be said: the tumor cells present showed changes in their form and arrangement and revealed atypical and asymmetrical mitoses. Therefore anaplasia, one of the most characteristic features of malignant tumors, most certainly explains some of the cellular variations encountered. As a matter of fact, Chambers² and other investigators interested in the grading of malignant tumors of the cervix use some of the variations of tumor cells here described as a basis for their grading. Yet it seems that the findings of islands of certain cell groups, morphologically seemingly entirely different from the rest of the tumor cells, in a single tumor, cannot be explained on the basis of ana-

plasia. Furthermore the presence of squamous and basal cells, both in a single malignant tumor and in obviously benign melanotic tumors, must be explained some other way. The other possibility is that the various cell types in a single tumor might perhaps be explained on the basis of "collision tumors." Collision tumor, according to Meyer,¹⁶ is a tumor consisting of two primary tumors which have invaded each other. If this were true of the tumors in this series it would be expected, in serial sections, to trace both tumors to their separate origins. No such two distinct tumors, however, were found. It also seems unwise to classify these tumors as "combination tumors," which are the result of the growth of two different blastomatous portions derived from one stem cell, as for instance, the Wilm's tumor of the kidney. In this type of tumor one would expect more evidence of mixed tumor characteristics than was actually encountered. "Composition tumors" as explanation can also be ruled out since this term signifies those tumors in which both parenchyma and stroma have become blastomatous. As there is no evidence in this series of an original tumor secondarily having changed its characteristics, it is fairly safe to assume that these tumors cannot be classified as "mutation tumors."

Ewing⁵ pointed out that carcinomas may sometimes vary greatly in morphology and may lead to much confusion of nomenclature. Malignant tumors, when altered by inflammation, hemorrhage, adaptation to mechanical environments and interference by surgical procedures, tend to assume indifferent structures in which most of the original features are lost and from which, as Ewing⁵ points out, it is usually hazardous to attempt to reach any conclusions regarding histogenesis. Because of this, and because of the recognition of cellular variations in individual tumors, it was thought wise to review critically reported instances of so-called collision and combination tumors. In a later study¹⁷ it is shown that a vast majority of reported carcinosarcomas are apparently single tumors. In some of these cases it seems very likely that reported carcinosarcomas really are squamous cell carcinomas with either basal cell or transitional cell features. This is particularly so in the so-called carcinosarcomas of the mouth, pharynx and esophagus.

Basal cells of the malpighian layer are often regarded as multi-potential cells. If this assumption were the correct explanation for

the variety of cells found in these tumors, in other words if basal cells under the influence of tumor forming factors were able to form squamous cells as well as pigment bearing cells, and also adenomatous structures, it would be expected that these various cells would be found in individual tumors regularly or at least much more frequently. However, these variations of tumor cells are by no means regularly encountered but are seen only in the occasional case.

The essence of this investigation is that a detailed study revealed that a number of single tumors not only consist of one type of tumor cells but of two or more morphologically different types of tumor cells. These tumor cells seem to be derived from cells which under normal conditions are found in the location subsequently taken up by the tumor. To trace well defined and differentiated cell groups to certain non-differentiated cells always seems hazardous. On the other hand, when basal cells and squamous cells are present within the same tumor it seems more likely that such a tumor originally started in basal cells and squamous cells. In instances where the tumor consists of glandular structures and squamous cells it seems likely that the tumor originally started in the region where both types of structures were present. It would appear that the findings here presented, namely the presence of various types of tumor cells, indicate that these tumors arose not from individual cells or certain cell groups but rather from one area which *a priori* contained the various cells of which the tumors consist, or derivations of these cells.

The practical application of this study seems clear. As far as grading of malignant tumors is concerned, the presence of the various cell types has been taken into consideration already (Chambers²). It is known that transitional cell carcinomas of the cervix, transitional cell carcinomas of the mouth, and basal cell tumors of the skin, and so on, are much more radiosensitive than squamous cell carcinomas. It is also established that the prognosis varies according to the type of cells present in the tumor. The type of radiation as well as the prognosis must be changed if a closer study reveals that what is reported as a one cell type tumor also contains other cell types. Naturally a transitional cell carcinoma of the oral cavity with squamous cell features will respond less readily to radiation than a simple transitional cell carcinoma.

Thus in this laboratory if various cell types are encountered the diagnosis of these tumors always states "predominating squamous cell carcinoma" or "predominating basal cell carcinoma," and so on. Basal cell carcinomas which recur after treatment and transitional cell carcinomas which are not as radiosensitive as others almost invariably reveal the presence of malignant squamous cells.

SUMMARY AND CONCLUSIONS

A study of miscellaneous carcinomas revealed morphological variations among the individual tumor cells of particular neoplasms. These morphological differences were not those always encountered in malignant tumors, but were often so heterogeneous as to obscure the exact nature of the tumor. They were principally brought about by the presence of seemingly different types of tumor cells in an individual tumor. Thus, basal cells or transitional cells were found in squamous cell carcinomas. The presence of various types of tumor cells in single tumors may be due to a number of factors which are discussed. However, the findings here presented seem to indicate that these tumors arose from one area which *a priori* contained the various cells of which the tumor consists, or derivatives of these cells.

Morphological variations must be taken into consideration in the grading of carcinomas and also in the determination of the radium sensitivity of tumors. The relative radium resistance of some basal cell or transitional cell carcinomas could be ascribed to the presence of many squamous cells in both of these tumors.

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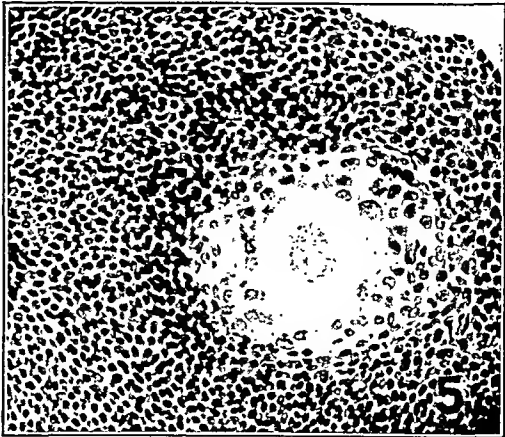
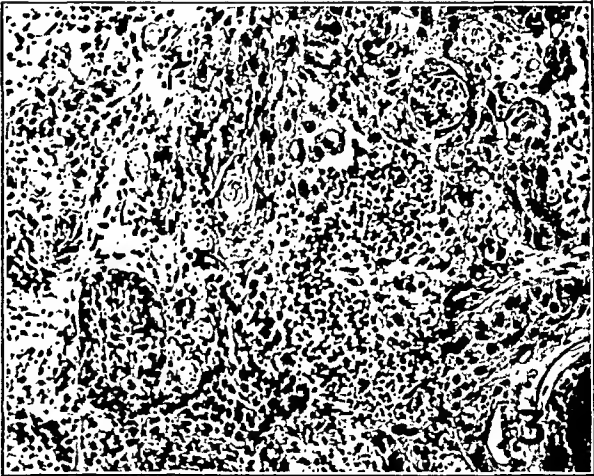
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DESCRIPTION OF PLATES

PLATE 123

- FIG. 1. Uterine tumor. Note the islets of squamous cells in an adenocarcinoma. Hematoxylin-eosin preparation. $\times 70$.
- FIG. 2. Adenoacanthoma of the uterus. Note the islet of squamous cell carcinoma in the middle of the picture. Iron hematoxylin-eosin preparation. $\times 70$.
- FIG. 3. Carcinoma of the cervix. Note the squamous and transitional cells. Iron hematoxylin-eosin preparation. $\times 95$.
- FIG. 4. Carcinoma of the skin. Note the squamous and basal cells. Hematoxylin-eosin preparation. $\times 80$.
- FIG. 5. Nevus of the skin. Note the squamous cells in addition to nevus cells. Hematoxylin-eosin preparation. $\times 135$.

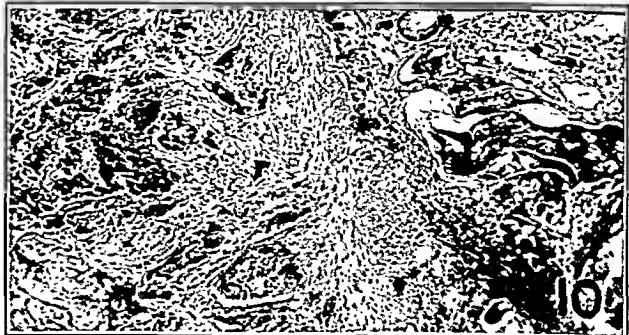
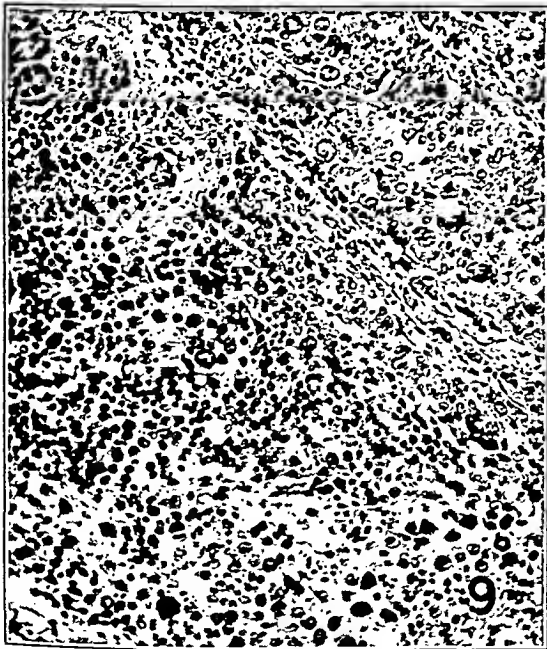
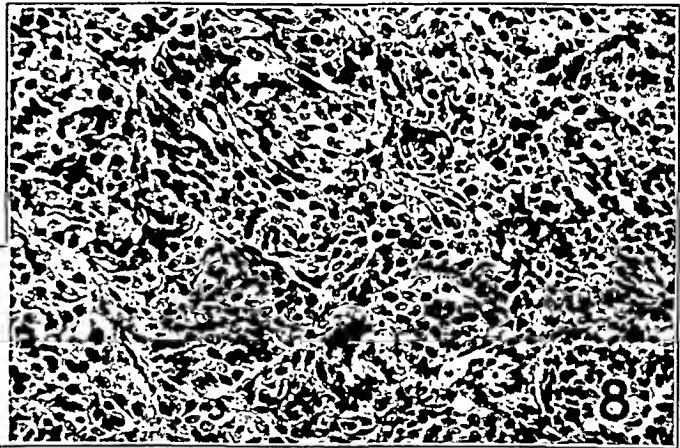
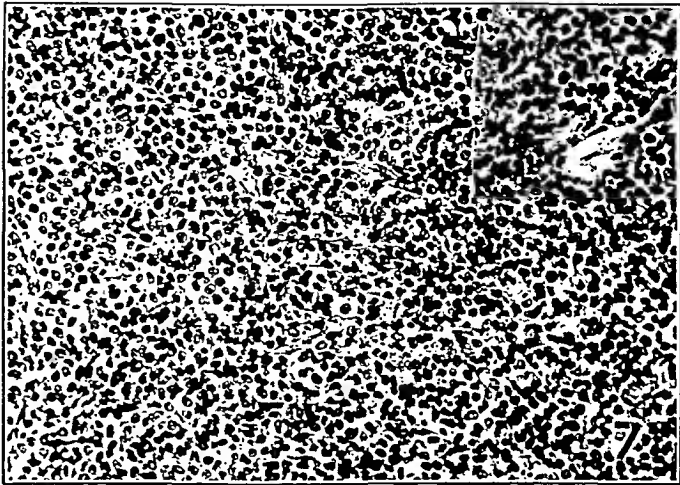
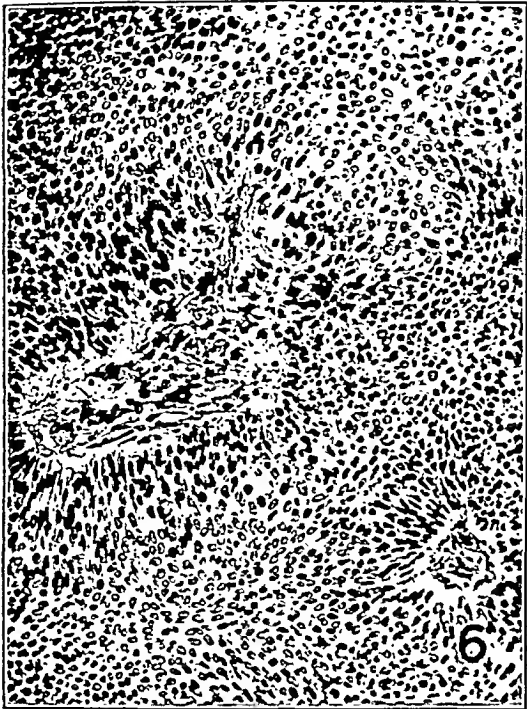


Saphir

Morphological Variations of Tumor Cells

PLATE 124

- FIG. 6. Nevus of the skin (same tumor as Fig. 5). Note the nevus cells in addition to the basal cell type. Hematoxylin-eosin preparation. $\times 135$.
- FIG. 7. Malignant melanotic tumor of the skin. Hematoxylin-eosin preparation. $\times 100$.
- FIG. 8. Malignant melanotic tumor of the skin (same tumor as Fig. 7). Hematoxylin-eosin preparation. $\times 100$.
- FIG. 9. Carcinoma of the mouth. Note the squamous cells in the right upper part of the field and the anaplastic transitional cells in the left lower portion. Hematoxylin-eosin preparation. $\times 90$.
- FIG. 10. Carcinoma of the rectum. Note the adenomatous structures containing mucinous material and the transitional cell type of tumor cells. Hematoxylin-eosin preparation. $\times 50$.



Saphir

Morphological Variations of Tumor Cells

THE MATRIX OF THE EPITHELIAL CELL INCLUSION BODY OF TRACHOMA *

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The epithelial cell inclusion bodies of trachoma were described by Halberstaedter and von Prowazek in 1907.¹ These investigators considered that the inclusions, as seen in Giemsa stained epithelial scrapings, were made up of two components: (1) a bluish staining ground substance or matrix which they called plastin; and (2) a mass of very fine, distinct reddish granules which they termed elementary bodies and regarded as the active agent of the disease. They believed that these elementary bodies multiplied by binary fission and increased at the expense of the ground substance which finally, in the mature inclusion, persisted only in the form of small irregular islands.

The interpretation of the blue substance as a matrix was contested by Lindner,² who showed by means of sections and wet-fixed preparations that what often appeared to be amorphous material in dry-fixed preparations was actually a compact mass of well defined, relatively large granular bodies. He studied these bodies, which he termed initial bodies, outside the cell and demonstrated division forms and forms showing transition to the smaller elementary bodies.

This type of morphological variation of the component bodies of cytoplasmic inclusions has since been recognized in two other virus diseases — inclusion blennorrhoea^{3, 4} and psittacosis.^{5, 6} In the latter a dense basophilic staining matrix, masking the component virus granules, was found to be demonstrable in immature inclusions and absent in well developed forms. Ordinary dyes have failed to demonstrate a matrix in the inclusions of inclusion blennorrhoea.

In 1936 Rice⁷ discovered that the inclusion bodies of trachoma

* This study was made in coöperation with the Health Division, Office of Indian Affairs, Department of the Interior, Washington, D. C.

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and inclusion blennorrhea stain a deep reddish brown with Lugol's solution and from his study he concluded that the inclusion body contained carbohydrate, probably glycogen, diffused through the inclusion to form a matrix. This matrix could be removed by diffusion or by digestion with saliva without affecting the component elementary and initial bodies.

The present study was undertaken in an attempt to confirm the existence of such a matrix. Material was obtained from trachoma cases in Indian children, from epithelial scrapings obtained in the United States Trachoma Hospital at Rolla, Missouri, and from trachoma cases at the Vanderbilt Clinic, New York City.*

THE IODINE STAINING OF TRACHOMA INCLUSION BODIES

Epithelial scrapings from active trachoma cases, both unfixed and fixed with absolute methyl alcohol, were stained with Lugol's solution (unmodified and diluted 1:2 and 1:4) by the method described by Rice. A drop or two of the solution was placed on a dry preparation, covered with a coverslip and examined in the wet state. The inclusion bodies appeared as deep reddish brown masses against a pale yellow background produced by normal epithelial cells, leukocytes and débris (Figs. 1, 2 and 4). Under low power magnification the iodine staining material was sometimes so dense as to appear almost homogeneous, but ordinarily it looked slightly reticular. Under oil immersion ($\times 1375$) this non-homogeneity was seen (Fig. 1) clearly to be due to innumerable minute clear areas, later found to correspond with the granular components of the inclusion, the non-staining elementary and initial bodies. There was excellent contrast between the inclusion mass and the yellow background, particularly in thick smears.

The inclusions could be seen also, though less well, in preparations in which the Lugol's solution had been allowed to dry.

When the iodine stained preparations were later stained with Giemsa and reexamined, there could be no doubt that the iodine staining material actually represented inclusions. Artifacts sometimes took the iodine stain but were easily differentiable in all cases. There were occasional, black amorphous masses which were believed to be carbonized cell material from the platinum spatula

* I am indebted to Dr. Polk Richards and Dr. C. E. Rice for material used in this report.

used in taking the scrapings. A slight stippling of the cytoplasm of the polymorphonuclear leukocytes occurred, due to the presence of slight amounts of glycogen, but it was never sufficient to cause confusion. Epithelial cells undergoing degeneration sometimes took a diffuse brown stain but it could always be distinguished from the characteristic reddish brown of the inclusion.

For comparison, inclusion-free material was prepared from 10 normal conjunctivae and from 25 cases of bacterial conjunctivitis of different types. No formations in these preparations took the iodine stain characteristic of the inclusion bodies.

✓ A COMPARISON OF IODINE AND GIEMSA STAINED PREPARATIONS

Epithelial scrapings from 107 cases of trachoma in Indian children were stained first by the iodine method — methyl alcohol-fixed slides flooded with undiluted Lugol's solution and examined in the wet state under a coverslip. On the mechanical stage and with a magnification of $\times 250$, each slide was examined for iodine staining inclusion bodies. When one was found the cell was marked on the stage so that it could be found again. After complete examination the slide was immersed in distilled water for an hour or more to remove the iodine and was then stained for from $\frac{1}{2}$ to 1 hour in Giemsa's solution, diluted 1 drop to 2 cc. of distilled water. It was then passed through 2 changes of 95 per cent ethyl alcohol and reexamined. When inclusion bodies were found they were noted on the mechanical stage and a comparison was made with the iodine stain findings.

There was a marked difference in the amount of iodine absorbed by different inclusions, but in general large inclusions stained densely (see Fig. 2), small ones lightly or occasionally not at all: a certain number showed up with the Giemsa stain which had been missed entirely by the iodine stain. Without exception these iodine-negative inclusion bodies were small and made up almost entirely of the large initial body granules. On the other hand, the iodine stain clearly demonstrated inclusions in masses of epithelium which would undoubtedly have been missed in a low power examination of a preparation stained by Giemsa's method alone. The Giemsa stain provides insufficient contrast for the successful demonstration of inclusions in cell masses. Figures 2-5 show the same cells stained first with iodine alone, then also with Giemsa.

THE RELATION OF THE IODINE STAINING MATERIAL TO THE ELEMENTARY AND INITIAL BODIES

There was no evidence obtained to indicate that the iodine staining substance was either identical with the elementary or initial bodies or closely related to them, as would be the capsular material of a bacterium. It could be removed by treatment with saliva without affecting the granules. Further, in 5 of the 107 cases there were numerous free elementary bodies, demonstrable with Giemsa's method, which the iodine stain failed to reveal under oil immersion examination.

THE PROBABLE GLYCOGEN NATURE OF THE IODINE-REACTING SUBSTANCE

Rice was of the opinion that the carbohydrate of the matrix was glycogen because of its microchemical reactions. In the absence of a method for obtaining sufficient amounts of material for direct chemical examination it seemed desirable to compare the microchemical reactions of the substance with those of a known glycogen. Trachoma preparations and impression smears of rabbit's liver known to contain large amounts of glycogen were therefore subjected to a series of tests, conducted under identical conditions for the two substances, with the following results:

(1) *Color Reaction*: The iodine reacting substance of the trachoma inclusion body and the liver cell glycogen stained the same shade of reddish brown with Lugol's solution.

(2) *Reaction to Heat*: An iodine stained trachoma preparation exhibiting numerous inclusion bodies was flooded with fresh iodine solution, covered with a coverslip and heated over an alcohol lamp. Examined while hot, the inclusions were found to have faded markedly. As the slide cooled the color returned. Liver cell glycogen preparations treated in the same way reacted identically.

(3) *Diffusion*: Material from a case of trachoma known to have abundant inclusions was placed in dilute Lugol's solution (1:10) on a slide without being allowed to dry. A considerable number of iodine staining inclusions was noted. The preparation was then placed in a moist chamber to prevent drying and re-examined at intervals of 3 and 4 hours. The color of the inclusions was seen to fade gradually and after 8 hours had disappeared.

The addition of fresh iodine did not restore the color, an indication that the carbohydrate had either been destroyed or dissolved out of the inclusion. The iodine reacting carbohydrate (glycogen) of a liver cell preparation disappeared in the same way under the same conditions.

(4) *The Effect of Drying on Diffusion:* Drying effectively prevented diffusion of the carbohydrate from the inclusion bodies. Dried preparations showing typical iodine staining inclusions were placed in distilled water for periods varying from several hours to 2 weeks. The apparent concentration of the carbohydrate, as judged by the intensity of the color reaction when the preparation was restained with iodine, was in no way affected. With one preparation the process of staining and decolorizing was repeated ten times without altering the reaction. Dried liver cell preparations retained their iodine staining carbohydrate under the same conditions.

(5) *The Effect of Saliva:* A dry unfixed preparation containing abundant inclusions was treated by dropping a small amount of saliva onto the slide. When it had been incubated for 1 hour, washed in water and restained with iodine, no color-reacting substance could be demonstrated. Liver preparations treated identically also lost their carbohydrate.

The test was repeated with saliva diluted 1:5 with normal saline. Half the diluted saliva was boiled to destroy the ptyalin, half left unheated. The trachoma preparations were placed upright in jars containing the two solutions and incubated for 4 hours. Those that had been treated with heated saliva still gave excellent color reactions; those treated with normal saliva gave none at all. This would indicate that the ptyalin itself was responsible for the disappearance of the carbohydrate. Liver preparations treated in an identical manner gave identical reactions.

(6) *Best's Carmine Stain for Glycogen:* Trachoma and rabbit's liver preparations were stained by the method of Best for the demonstration of glycogen. Both the glycogen in the liver cells and the carbohydrate in the inclusion bodies stained well. There was a variation in the degree of staining among the inclusion bodies according to size, the large inclusions staining most intensely.

The results of these several tests would seem to establish the matrix of the trachoma inclusion as glycogen or largely glycogen.

OTHER OBSERVATIONS ON THE MATRIX

A large number of inclusion bodies stained with Giemsa's method were examined for evidence of a Giemsa staining matrix. None was found. Certain inclusions showed a slight bluish veil over the elementary and initial bodies but this was probably due to the staining of the cytoplasm remaining above and below the inclusion. Preparations made by the wet method of fixation, in which the approximately normal shape of the cell is maintained, were also studied. Stained by Giemsa's method they revealed no staining matrix whatsoever. The elementary and initial bodies were seen to be embedded in what appeared to be a cytoplasmic vacuole which was perfectly transparent as compared to the blue of the unaffected cytoplasm (Fig. 6). Other stains, including neutral red, brilliant cresyl blue and hematoxylin, stained the elementary and initial bodies but not the matrix.

That the matrix has some cohesive properties was indicated by the finding of several intact inclusion bodies which had been extruded from cells. In general, however, there appeared to be wide scattering of the granules after destruction of the cell.

DISCUSSION

The findings in this study corroborate Rice's opinion that the inclusion body of trachoma contains a matrix in which the elementary and initial bodies are embedded and which is composed in large part of a carbohydrate having the microchemical properties of glycogen. The formation of the glycogen may be associated with the conversion of initial bodies into elementary bodies. One preparation which failed to reveal any iodine staining inclusion bodies with Lugol's solution exhibited many initial body inclusions when stained by Giemsa's method. A careful examination disclosed that these inclusions were all in an early stage of development and contained few, if any, elementary bodies.

The chemical composition of the ground substance of cytoplasmic inclusions would seem to vary. The fowl-pox inclusion, for example, is known to contain considerable lipoidal material.^{8, 9} The inclusion blennorrhea virus inclusion appears to be identical morphologically with the trachoma inclusion and at the present time is the only other one known to have a carbohydrate matrix.

The inclusion bodies of psittacosis and trachoma have been found by Bedson and the writer to bear a very close resemblance in that the component granules have the same morphological and tinctorial variation and cannot be distinguished from each other when free outside the cells. On the other hand Bedson has been unable to demonstrate a carbohydrate-reacting substance in the psittacosis inclusion; its matrix appears to be associated with the large form of the virus (initial body) and stains densely with Giemsa's solution and other dyes.

Certain workers are of the opinion that the elementary bodies of trachoma should be grouped with the Rickettsiae in view of their large size and parasitic nature and it will be recalled that the minute bodies of psittacosis were at one time described by Lillie¹⁰ under the name *Rickettsia psittaci*. The occurrence in the trachoma inclusion of a stainable matrix, characteristic of viruses but unknown to Rickettsiae, may be of importance in deciding this question. On the other hand, it is true that the elementary bodies of trachoma, inclusion blennorrhoea and psittacosis differ sharply in their ease of staining, morphological variation, type of inclusion body and obvious parasitic nature from the typical virus elementary body as represented by the Borrell bodies of fowl-pox and the Paschen bodies of vaccinia.

SUMMARY AND CONCLUSIONS

✓ The epithelial cell inclusion body of trachoma contains a ground substance or matrix which is composed predominantly of glycogen. This matrix is apparently absent or in low concentration in young inclusions and uniformly present in mature inclusions. Its formation seems to be associated with the change from the large, initial body form of the virus to the small, elementary body form. In view of the ease with which the inclusion bodies may be recognized with low magnification, the iodine stain should prove distinctly valuable as a laboratory test in the diagnosis of trachoma. ✓

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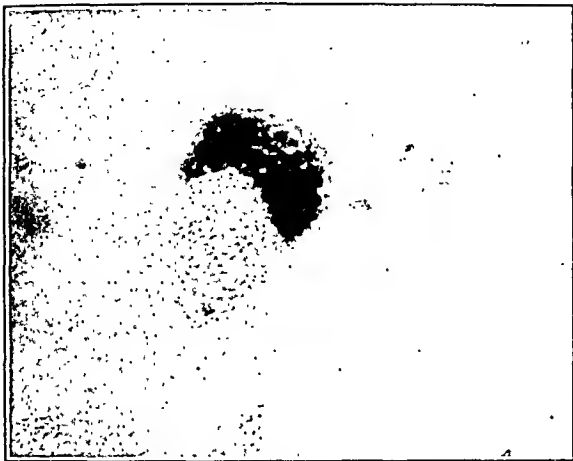
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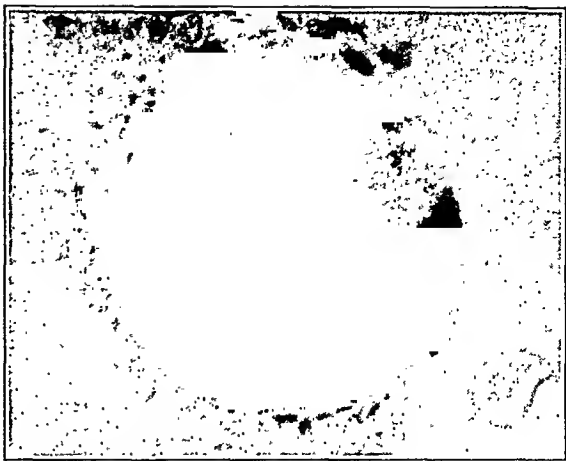
DESCRIPTION OF PLATE

PLATE 125

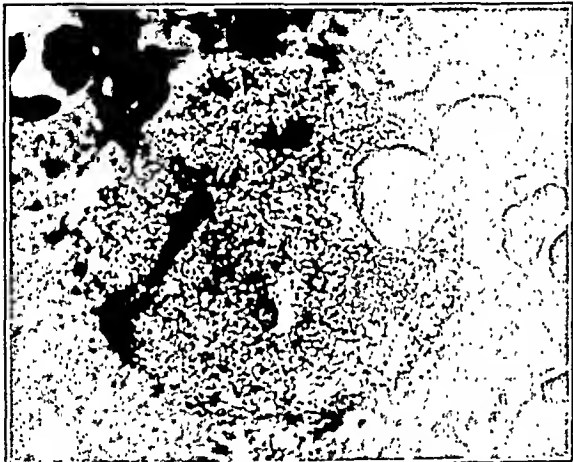
- FIG. 1. Epithelial cell inclusion stained with Lugol's solution and showing non-homogeneity of the matrix. $\times 2000$.
- FIG. 2. Large inclusion body occupying the entire cytoplasm of the epithelial cell. Stained with Lugol's solution. $\times 2000$.
- FIG. 3. The inclusion body shown in Figure 2 restained by Giemsa's method after removal of the iodine with water. It is seen to be composed of myriads of elementary bodies. $\times 2000$.
- FIG. 4. A large inclusion body staining densely with Lugol's solution. $\times 2000$.
- FIG. 5. The inclusion body shown in Figure 4 restained by Giemsa's method after removal of the iodine. $\times 2000$.
- FIG. 6. A wet-fixed (sublimite-alcohol) preparation stained by Giemsa's method and showing two inclusion bodies. There is no staining of the matrix and the elementary and initial bodies appear to be in a cytoplasmic vacuole. $\times 2000$.



1



2



3



4



5



6

Thygeson

Epithelial Cell Inclusion Body of Trachoma

THE ABILITY OF LYMPH TO MAINTAIN VIABILITY IN "DEVASCULARIZED" LYMPH NODES *

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In the course of experimental work concerning the physiology of the lymphatic system it occurred to the authors that some light might be shed on the function of lymph if it could be shown that lymph *per se* could keep tissues alive. In the following experiments all vascular connections to the popliteal lymph nodes of dogs were severed, but one or more afferent and one or more efferent lymphatic channels were left intact and it was found that lymph sufficed to maintain viability. These results imply a nutritive function on the part of lymph which, so far as we are aware, has not been demonstrated *in vivo* in mammals.

METHODS

Normal, healthy, adult mongrel dogs maintained on kennel diet and allowed water *ad lib.*, were used as experimental animals. Under ether or nembutal anesthesia the popliteal node was exposed through a linear 4-6 cm. incision. Sterile technique was observed in all operative procedures.

In the *control* group of animals the node was completely excised with a minimum amount of hemorrhage and trauma and replaced in the popliteal space where it was held by suturing the subcutaneous fascia and skin over it. Attempts were made to ligate all severed afferent and efferent lymphatic channels as well as blood vessels.

In the *experimental* group all tissue which could not definitely be identified as lymphatic trunks was severed and these remaining trunks were cleaned as thoroughly as possible of any connective tissue that might carry blood capillaries. With experience one learns to identify lymphatic trunks with certainty by their translucency and the characteristic fashion in which they "bead" below

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a temporary obstruction. Especially is the identification of lymphatic trunks certain when a poorly diffusible dye (approximately 1 per cent Chicago blue or pontamine blue dissolved in saline, or preferably dog serum) is injected into the subcutaneous tissues between the toes, and the foot gently massaged. This procedure, which can be carried out before the operation is begun, or by an assistant after the operative field is exposed, almost immediately outlines the popliteal node and its afferent and efferent channels in sharp contrast. These dye injections were made in most instances in the experimental group, but in a number of experiments they were purposely omitted to exclude the possible (antiseptic?) effect of the dye.

At varying intervals after the operation the nodes were removed by biopsy or at autopsy, fixed in Zenker's solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, the elastic tissue method followed by van Gieson's counterstain, and Foot's modification of Bielschowsky's silver stain. In several instances Gram and methylene blue stains were also made.

In obtaining samples of afferent and efferent lymph for chemical analysis the technique described by Drinker and Field¹ was used. Reducing substance was determined by the Hagedorn-Jensen method²; bound carbon dioxide and carbon dioxide combining power by a volumetric method.*

EXPERIMENTAL OBSERVATIONS

The results are summarized in Table I. In the control group, *i.e.* dogs with all blood vessels and lymphatics severed, all 15 nodes rapidly underwent massive necrosis, as illustrated in Figure 1. These necrotic changes, which affected primarily the lymphocytic elements but also involved the reticulum and blood vessels, were maximal by 36 hours, following which the nodes underwent liquefaction, reabsorption and fibrous tissue replacement. It was difficult to follow these changes longer than 3 days as the nodes usually became infected and tended to slough out; in fact, 7 experiments were discarded because the node could not be identified in the depths of the open wound and no remnants were recognizable in histological sections of regional tissue. The almost constant presence of infection in this group at first made us suspect our oper-

* The authors are indebted to Dr. Joseph Victor for carrying out these analyses.

ative technique, but from histological studies of these nodes and especially from bacteriological studies of nodes obtained from normal dogs sacrificed for other purposes, we are convinced that in apparently normal healthy dogs, organisms (*Staphylococcus albus* and *aureus*, hemolytic and non-hemolytic streptococcus) are continually being filtered out of subcutaneous lymph by peripheral lymph nodes. The presence of necrotic tissue, which serves as a favorable medium for the growth of bacteria already present in the nodes, plus the absence of the normal defensive mechanism offered by the lymphatic system, affords an ample explanation for the high incidence of infection in this control group.

In the *experimental* group, *i.e.* dogs with all vascular connections severed but with one or more afferent and one or more effer-

TABLE I

Maintenance of Viability in "Devascularized" Lymph Nodes

Group	Interval between operation and biopsy	Number of nodes	Number of nodes completely necrotic	Number of nodes partially viable	Number of normal nodes	Number of infected nodes
Control *	days 1-3	15	15	0	0	15
Experimental †	1-9	20	2	14	4	3

* All vascular and lymphatic connections severed.

† All vascular connections severed, but one or more afferent and one or more efferent lymphatic channels left intact.

ent lymphatic channels remaining intact, the results, though not wholly consistent, are suggestive. Some degree of viability was maintained in 18 of 20 experiments (90 per cent); 4 of the nodes remained grossly and histologically normal (Fig. 2), and only 3 became infected. The explanation for the varying degrees of viability illustrated in Figures 3 and 4 is not clear; possibly mechanical and anatomical factors are responsible, such as kinking, thrombosis and leakage from lymphatic trunks, occlusion of trunks by pressure from without as by edema or fibrin, variation in the number and anatomical arrangement of trunks remaining patent, and variation in the amount of movement of individual dogs and the consequent variation in lymph flow through the nodes. The only evidence we have to support the suspicion that the number of patent channels is important is that when deliberate efforts were made to leave a single afferent and a single efferent channel intact so quantitative chemical studies could be done, all of the nodes

showed some anatomical change but none of them showed the uniform necrosis observed in the control group.

Having found a distinct difference between the experimental and control groups, and having shown that in 4 cases integrity of the node had been maintained, attention was next directed at showing that all the blood supply had been excluded. Three methods were used as follows:

Under nembutal anesthesia the left popliteal node was exposed, all blood vessels severed, and its afferent and efferent lymphatic channels freed for a distance of about 3 cm. so that a rubber tissue dam could be interposed between the node and the underlying tissues. In this way the lymphatic channels entering and emerging from the node could be observed directly at any time during the experiment. The right popliteal node was then excised and placed on the rubber tissue dam beside the experimental node. Both were covered with a thin gauze pad kept moist by a warm saline drip and maintained at a temperature of 35–38 degrees C. by the use of lights. The left hind leg was then secured above the ankle and the foot moved passively with a mechanical device to ensure continuous lymph flow. The animal was maintained under light anesthesia by repeated small subcutaneous injections of nembutal. At 10:30 P.M., 12 hours after the experiment was started, dye was injected into the subcutaneous tissues between the toes of the left hind foot. The afferent channels, the node and the efferent channels became filled with dye almost immediately. Dye injection was repeated just before the experiment was terminated at 8:30 the following morning. By this time gelatinous thrombi had formed in some of the afferent trunks but by massaging upward along the course of the lymphatic trunks from the foot dye could be forced past the node into the efferent channels. At no time during the course of the experiment were any blood capillaries seen coursing along the lymphatic trunks, although these were looked for at frequent intervals. Section of the control right popliteal node showed massive complete necrosis with early liquefaction. The experimental node, however, showed only slight early necrosis in a few places and this may well be related to trauma, "saline extraction," or to the terminal thrombi.

In another experiment the node was enclosed in a rubber dam. This satisfactorily excluded the possibility of granulation tissue

growing through the capsule save along the afferent and efferent lymphatic trunks at the poles of the node which, of course, penetrated the rubber sheath. The experimental node appeared normal when it was excised 7 days later. The opposite popliteal node, which was completely removed and replaced in the popliteal space enclosed in a rubber sheath, showed massive necrosis and infection when it sloughed out 3 days later.

The deprivation of vascular supply was also confirmed by intra-arterial injection of India ink. One to 3 days after isolating the popliteal node on one side, save for its lymphatic connections, the dog was anesthetized and a cannula placed in each femoral artery, and tourniquets placed about 5 cm. above and below each "knee." Saline (about 150 cc.) was then perfused through the arteries until the fluid that returned through the cut end of the femoral veins was clear. Full strength Higgins' India ink (about 30 cc.) was then run through the cannulas until the returning fluid was black. Whereas practically every blood vessel in the node on the unoperated side was distended with ink, of the 6 devascularized nodes on which this procedure was tried ink was found in only one small arteriole in 1 node. The necrotic changes in this node were as great as in the other 5 nodes which contained no ink.

Evidence from all three types of experiments indicates that the blood supply to the nodes had been excluded. Any nourishment to the nodes, therefore, must have come by way of the lymphatic trunks or by diffusion through the capsule from the surrounding tissues. That the latter mechanism is totally inadequate to maintain the vitality of the nodes is evident from the uniform necrosis in the control group. Nutritive material must have reached the nodes through the lymphatic channels.

In a limited number of experiments, sugar, bound carbon dioxide, and carbon dioxide combining power determinations were made on afferent and efferent lymph obtained by cannulating lymphatic trunks leading to and from these "devascularized" nodes. The results of 3 experiments are given in Table II.

The data in Table II indicate certain relations between glucose disappearance and decrease in both carbon dioxide and carbon dioxide combining power of lymph during its passage through the lymph nodes. The mean decrease in lymph carbon dioxide and carbon dioxide combining power was 12-13 volumes per cent re-

TABLE II
Chemical Analyses of Lymph Flowing to and from "Devascularized" Lymph Nodes

Number of dog and node	Number of hours after severance of blood vessels	Lymph flow through node †	Sugar		Carbon dioxide				Histology of node
			A	E	Bound		Combining power		
					A	E	A	E	
			mg./ 100 cc.	mg./ 100 cc.	vol./%	vol./%	vol./%	vol./%	
37-144 (RPN)	2½	0.45	116	59	38	27	61	48	Normal
37-145 (RPN)	3½	1.05	109	66	41	31	61	48	Normal
37-145 (LPN)	24	1.40	146	110	45	30	58	45	Slight necrosis of medulla, follicles viable

A = afferent lymph; E = efferent lymph; RPN = right popliteal node; LPN = left popliteal node.
 These figures represent amount of efferent lymph collected from a single trunk.

Slight necrosis of medulla, follicles viable

spectively, which is equivalent to 48–52 mg. of lactic acid. This indicates that under the conditions of this experiment all of the glucose that disappeared can be accounted for by its conversion to lactic acid. With intact blood supply there is no evidence of decrease of glucose from the lymph.³ Either glucose is supplied to the node directly by the blood or else the loss from the lymph is masked by diffusion replacement from the blood. Under aerobic conditions lymph nodes from mice placed in Ringer's solution produce very little lactic acid from glucose but considerable quantities may be produced anaerobically.⁴ Normal lymph nodes also oxidize glucose. Therefore, since under the conditions described above the changes in lymph glucose and lymph carbon dioxide and carbon dioxide combining power are equivalent, they indicate that during the period in which the *lymph gland remains viable its metabolism is mainly anaerobic*. This is not surprising when it is recalled that the rate of oxygen consumption of lymphoid tissue of the mouse is about 1.02 cc. per gm. per hour.⁴ If the oxygen content of afferent lymph is equal to that of plasma, then 1 cc. would contain about 5 cmm. of oxygen. Since the rate of lymph flow through a node is about 3 cc. per gm. per hour,* the lymph could supply only about 1 per cent of the oxygen required for respiration.

The obvious step from these *in vivo* experiments, which amount to nothing more than perfusion with lymph, to experiments *in vitro* in which the nodes are perfused with "artificial lymph" and in which there is no question of persistent blood supply, has been taken. The results of early experiments confirm the *in vivo* studies listed in Table II in all respects. These results will be reported later. Suffice it to state here that the chemical studies to date have yielded confirmatory evidence that the blood supply to the nodes has been severed and have indicated that anaerobic glycolysis is one of the metabolic processes effective while they are maintained in a viable state.

DISCUSSION

When it is taken into consideration that lymph was one of the first substances used as a tissue culture medium and that the com-

* This figure is based on an average weight of 1.5 gm. for popliteal lymph nodes from dogs, an average flow of 1.5 cc. per hour per lymphatic trunk,⁵ and an average of three afferent trunks.

position of the plasma-Tyrode mixture commonly employed today for tissue culture work closely approximates that of lymph in glucose, electrolyte and protein content, and further that bland infarction of lymph nodes is a rarity, if it ever occurs, the observation that lymph *per se* will maintain the viability of lymph nodes is not surprising. But does this observation give an indication as to the normal function of lymph?

The great bulk of evidence today indicates that lymph, at least in the peripheral portions of the body, is derived from the blood plasma. The rate of lymph formation and the constant presence in the lymph of appreciable quantities of proteins indistinguishable from those in the blood plasma render any other interpretation untenable. Without entering into the controversy as to the identity of tissue fluid and lymph, it is fair to state that all workers are agreed that the fluid which flows through the lymphatic channels represents that portion of the blood plasma filtrate not reabsorbed by the blood capillaries and not utilized by the body cells which are bathed by it before it enters the endothelial lined channels of the lymphatic system to become lymph. That the body cells may add to as well as subtract from the tissue fluid and that all these changes may alter the composition of lymph is also generally recognized. The experimental observations in this paper indicate that this fluid, which has been "rejected" by certain tissues of the body, is still capable of maintaining viability in other tissues. The simplest explanation for this observation is that the excess nutritive material is merely passed on. The magnitude of this "factor of safety," however, is not great* and it is possible that qualitative as well as quantitative factors are involved. How long such nodes can be maintained free from blood supply, whether any morphological changes in the node will occur with time, and

* If the amount of tissue whose lymph drains into the popliteal node of a dog weighing 10 kilos be estimated at 200 gm., and if a pulse rate of 100 and a cardiac output of 30 cc. per beat be assumed, and further if the assumption be made that each gram of tissue in the dog receives the same amount of blood, it can be calculated that the blood flow to the 200 gm. of tissue is 3600 cc. per hour. The total amount of lymph flowing through the node, even under conditions of activity, probably would not exceed 10 cc. per hour; from statistical analysis⁶ the actual lymph flow would be nearer 5 cc. per hour, a factor of safety of about 0.2 per cent. On the same admittedly crude basis of computation, the blood flow through the node (assuming a weight of 1.5 gm. for the node) would be 27 cc., or about six times the lymph flow. The actual blood flow through the lymph node is probably much greater.

whether such nodes are capable of hyperplasia or antibody production in response to appropriate stimuli are all questions for future investigation.

SUMMARY AND CONCLUSIONS

Popliteal lymph nodes of dogs when replaced in the popliteal space after complete severance of all vascular and lymphatic connections rapidly undergo massive necrosis. These nodes usually become infected and may slough out.

When, however, all vascular connections are severed but one or more afferent and one or more efferent lymphatic channels remain intact, infection does not ensue and the nodes remain viable. Chemical analyses on lymph flowing to and from these "devascularized" nodes show a sharp drop in reducing substance, bound carbon dioxide and carbon dioxide combining power in the lymph during its passage through the node, and indicate that anaerobic glycolysis is one of the metabolic processes taking place in the viable node.

These observations imply a nutritive function on the part of lymph which, so far as we can determine, has not been demonstrated *in vivo* in mammals.

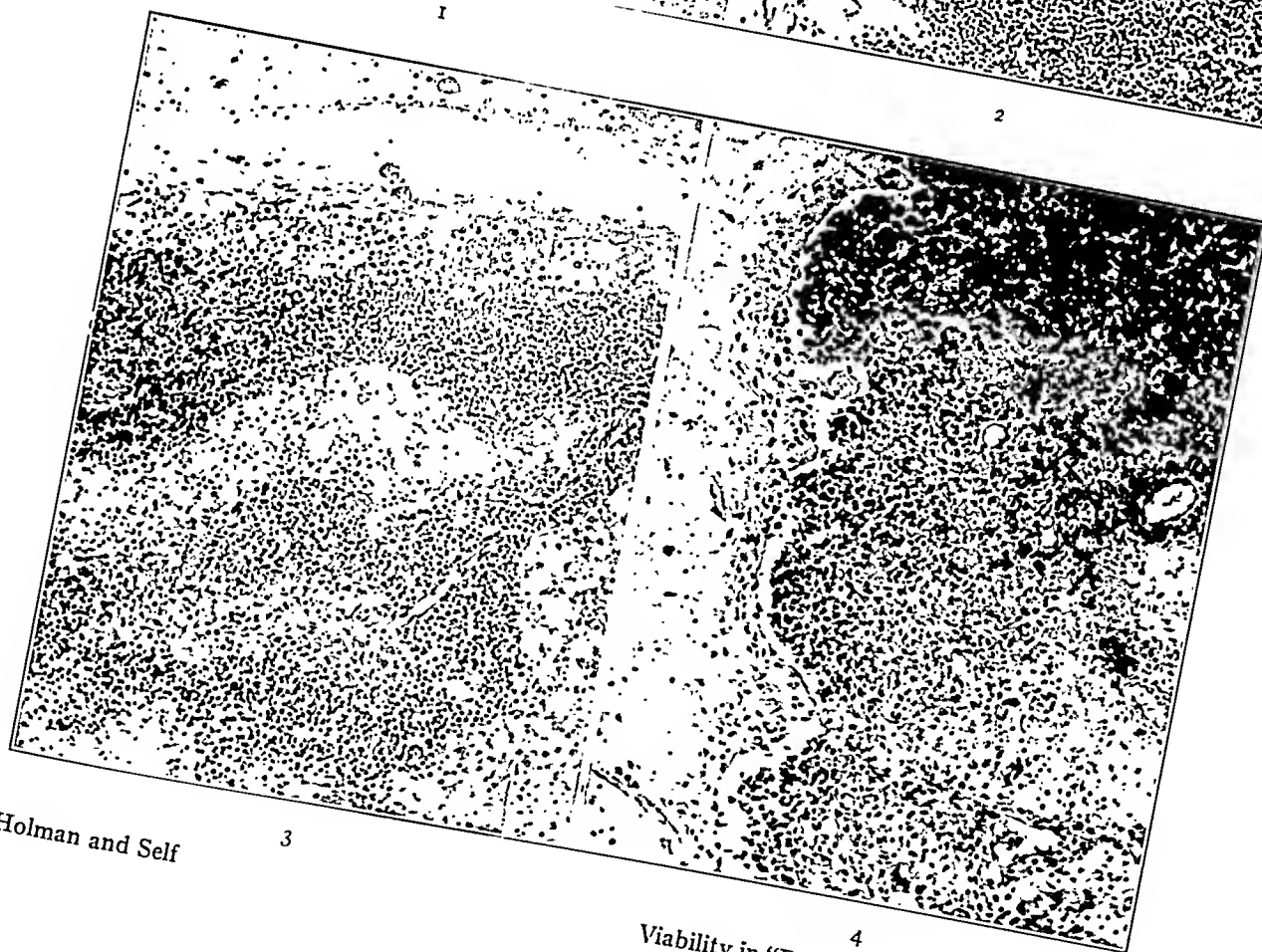
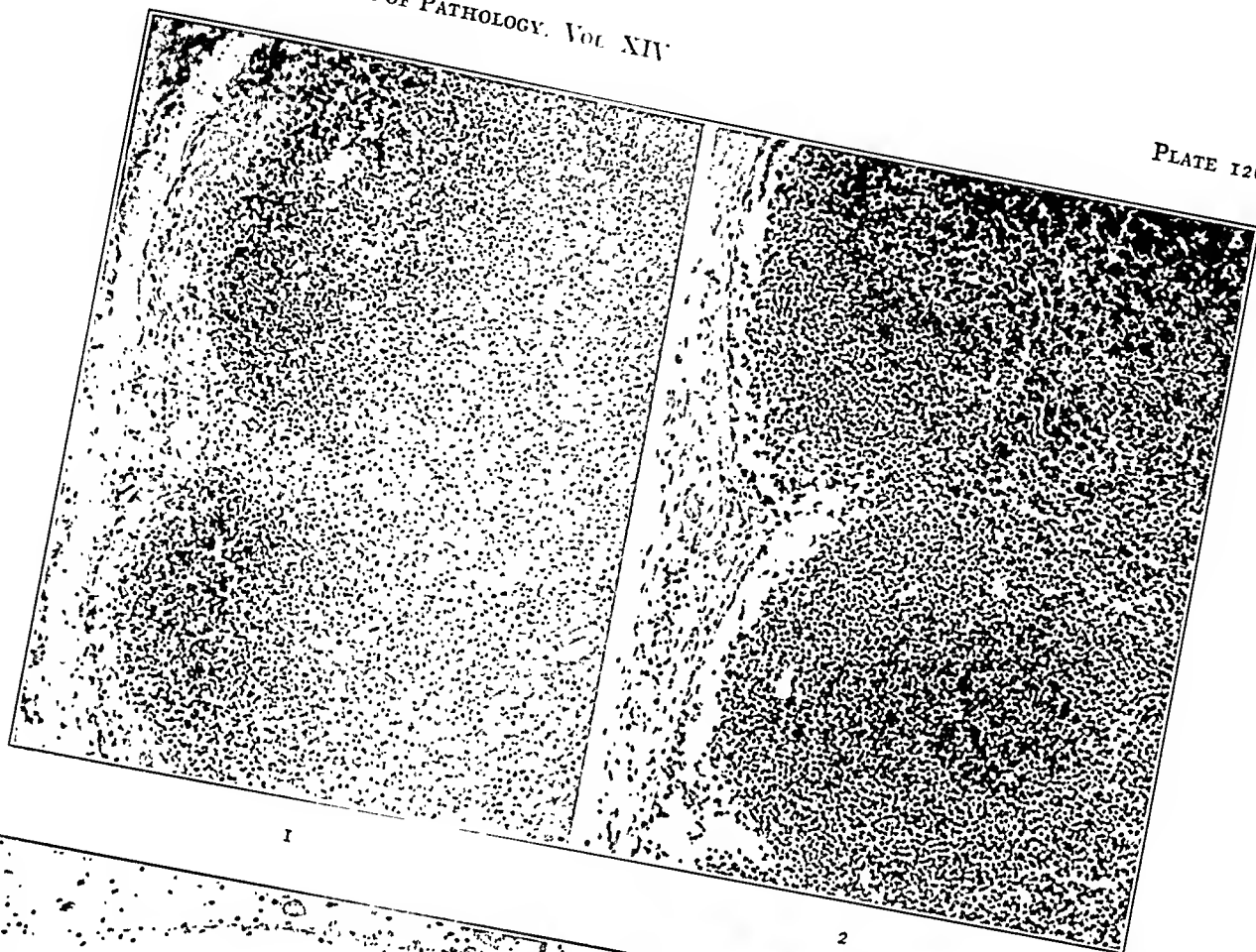
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DESCRIPTION OF PLATE

PLATE 126

- FIG. 1. Complete necrosis in lymph node 29 hours after severance of all lymphatics and blood vessels.
- FIG. 2. Normal structure of node preserved 7 days after severance of blood vessels; lymphatics left intact.
- FIG. 3. Partial viability in node 1 day after severance of blood vessels; lymphatics left intact.
- FIG. 4. Partial viability in node 2 days after severance of blood vessels; lymphatics left intact.



Holman and Self

Viability in "Devascularized" Lymph Nodes

THE RESIDUAL INFECTIVITY OF THE PRIMARY COMPLEX OF TUBERCULOSIS *

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Whether or not the lesions constituting the primary complex of tuberculosis ultimately heal, in the sense that the morbid focus no longer contains virulent organisms of *Mycobacterium tuberculosis*, has been investigated and discussed by many workers since the report of Dejerine¹ in 1884. While morphological criteria frequently suggest that the infective agent in the childhood type of tuberculosis persists for long periods of time, the question cannot always be determined with definiteness unless it can be demonstrated that material from the lesions is infective for guinea pigs. The problem is of clinical significance since the persistence of apparently quiescent or healed tuberculous foci within the body is a definite menace as long as virulent tubercle bacilli remain.

REVIEW OF THE LITERATURE

Dejerine¹ in 1884 was among the first to investigate this problem. He examined caseous and calcified lesions of tuberculosis from 12 subjects who were in the fourth, sixth, seventh and eighth decades of life and failed to find the tubercle bacillus in tubercles that were completely calcified. In tubercles that were completely calcified in the center with traces of caseation at the periphery, the bacilli were found in smears or histological preparations from the peripheral portions but not from the center. Material from 4 of the subjects whose lesions were completely or incompletely calcified was used to inject guinea pigs. The results were all considered negative at the time his paper was published, although the experiments on incompletely calcified nodules were considered as unfinished. Dejerine concluded that when calcification of a lesion is complete the infective agent has disappeared and the lesion may be considered as healed. Kurlow,² as a consequence of the results

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obtained following the injection of animals, considered tuberculous lesions that were caseated to be potential sources of reinfection, and that for a lesion to be entirely healed it must be completely calcified. Birch-Hirschfeld³ studied the incidence of tuberculosis among 826 persons who had died from accidents or from acute diseases and found tuberculous lesions in 171, or 20.7 per cent. Of those showing anatomical evidence of tuberculosis, the lesions in 105, or 61.4 per cent, were considered as healed; lesions in 31, or 18.1 per cent, appeared to be definitely active; and those in 35, or 20.5 per cent, were presumed to be latent or mildly active.

Rabinowitsch⁴ tested the infectivity for guinea pigs of calcified tuberculous lymph nodes from 4 adults, 1 of whom had pulmonary tuberculosis. Positive results were obtained in each instance. Weber,⁵ by the injection of guinea pigs, studied a series of 39 children less than 15 years of age who had primary tuberculosis of the intestine or mesenteric lymph nodes. Material from 17 of these children whose disease was limited to the mesenteric lymph nodes failed to produce tuberculosis in the experimental animals. The lesions in these 17 cases were caseous and calcified.*

Schmitz⁶ injected guinea pigs with material prepared from calcified tuberculous foci from 28 subjects and obtained positive results in 13 instances. The material studied consisted of foci from the lungs and from the tracheobronchial and mesenteric lymph nodes.

Bugge is quoted by Hektoen⁷ as having found that 35 per cent of 138 subjects more than 1 year of age who had died from causes other than tuberculosis showed morbid changes that were interpreted as representing healed lesions of tuberculosis.

Opie⁸ reported the results of a study designed to determine the incidence and the structural character of lesions of tuberculosis in the lungs of children and adults. Opie's search for lesions was especially thorough, since in addition to the usual dissection methods the different lungs were examined roentgenologically. Of the 93 children examined, lesions of tuberculosis were found in the lungs of 22. Eleven had died of tuberculosis. Of 50 adults examined, tuberculosis was the cause of death in only 3 instances,

* The bovine type of *Mycobacterium tuberculosis* was present in 13 of the 22 cases in Weber's series in which the results were positive. In 7 cases the human type of the organism was demonstrated, and in 2 both the bovine and the human types were present.

yet tuberculous lesions were found in the lungs of all. Morphologically lesions that were firmly calcified in the center and had a capsule of fibrous connective tissue were considered as healed. When young tubercles were present the lesions were considered as active. Opie emphasized the importance of calcification as indicative of the fact that a lesion had healed. He excluded from the group in which the lesions were considered as healed those in which calcification was not complete and those in which caseous lesions were found within the regional lymph nodes. Morphologically Opie classified the lesions in the lungs of the 22 children as follows: healed in 3, caseous and encapsulated in 6, and active in 13. The lesions in the adults were classified as healed in 19, caseous and encapsulated in 25, and active in 6.

In a later study Opie,⁹ in collaboration with Aronson, examined material from 97 lungs and 178 lymph nodes from 169 individuals with the childhood type of tuberculosis. The material was used to inject guinea pigs and, when the results obtained from examination of the lungs and lymph nodes were considered together, it was found that living organisms of *Mycobacterium tuberculosis* were present in the different types of lesions as follows: in caseous lesions that were partially fibrotic, 33.3 per cent; in caseous encapsulated lesions, 23.3 per cent; in caseous calcified lesions, 4.4 per cent; and in calcified nodular lesions, 20 per cent.

Opie and Aronson⁹ thought it was not improbable that the positive results obtained for guinea pigs that were given injections of material from calcified nodules of the lungs and from the lymph nodes might have resulted from the presence of tubercle bacilli in the tissue surrounding the morbid focus rather than from bacilli within the substance of the focus. To determine the validity of this hypothesis guinea pigs were given injections of fairly large amounts of material prepared from the apices and bases of lungs and from the contiguous lymph nodes of 33 persons. Macroscopically none of the tissues used for injection appeared to be tuberculous although lesions of chronic tuberculosis were present elsewhere in the lung. The results of animal inoculation tests revealed that living organisms of *Mycobacterium tuberculosis* were present in one or the other of the tissues examined in 15 of the 33 cases. The authors concluded, therefore, that the development of tuberculosis in guinea pigs given injections of material from case-

ous encapsulated or calcified nodules of the lung or lymph nodes is due with few exceptions to the presence of tubercle bacilli in the tissues surrounding the focus and not in the focus itself. They believed that, generally speaking, the organism of tuberculosis disappeared from the lesions when calcification became evident.

Griffith¹⁰ in 1929 reported on the types of *Mycobacterium tuberculosis* that had been demonstrated in tuberculous lesions of human beings and included data on a considerable number of cases in which lesions of tuberculosis presumably were present but in which living tubercle bacilli could not be demonstrated by the inoculation of guinea pigs. Of 319 cases investigated there were 47 with gross lesions of tuberculosis in which no living organisms of *Mycobacterium tuberculosis* could be found. Griffith gave a summary of 178 "instances" * in which tissues containing tuberculous lesions had failed to be infective for guinea pigs, although acid-fast forms resembling those of *Mycobacterium tuberculosis* were present. Cultures were attempted in 97 instances, with negative results. Griffith's observations indicated that tuberculous lesions of long duration rarely if ever contain living tubercle bacilli. He found healed lesions in persons ranging in age from 11 months to 70 years and concluded that lesions of human tuberculosis "whether produced by human or by bovine tubercle bacilli and wherever situated, may undergo spontaneous arrest and cure."

Robertson¹¹ in 1933 reviewed the problem of the persistence of tuberculous infections in human beings and mentioned the relative frequency of cases in which the latent or dormant characteristics of tuberculous infections could be demonstrated morphologically. He reviewed a series of 3306 consecutive autopsies and found evidence of tuberculosis in some form in 2064 (62.43 per cent). In 89 of the cases tuberculosis was either the principal or a contributing cause of death, and in 1725, changes that were considered to represent healed lesions of tuberculosis were found. By morphological criteria such as proliferation of connective tissue, giant cells and accumulations of lymphocytes, Robertson classified the lesions in 134 (4.05 per cent) of the cases as active. He concluded, in part, that tuberculous infections may subside and be regarded as healed, yet remain continuously active, and that tuberculous

* Whether or not the word "instances" represented 178 separate cases was not clear.

lesions, which have apparently healed, may become clinically active after varying intervals.

Hektoen also expressed the opinion that infection with *Mycobacterium tuberculosis* persists for a prolonged period. He maintained that caseous material, such as occurs in tuberculous lesions, is not readily absorbed and that the bacilli may persist even when such lesions are completely encapsulated. He stated that partly calcified sclerotic areas "containing caseous or calcareocaseous foci cannot be regarded as completely healed but rather as having passed into a state of latency." Hektoen considered a tuberculous lesion as healed only when there had been formed a more or less structureless mass, with or without the deposit of calcium and without caseous material being present. He expressed the opinion that caseous material, even of long duration and partially calcified and encapsulated, may contain virulent tubercle bacilli.

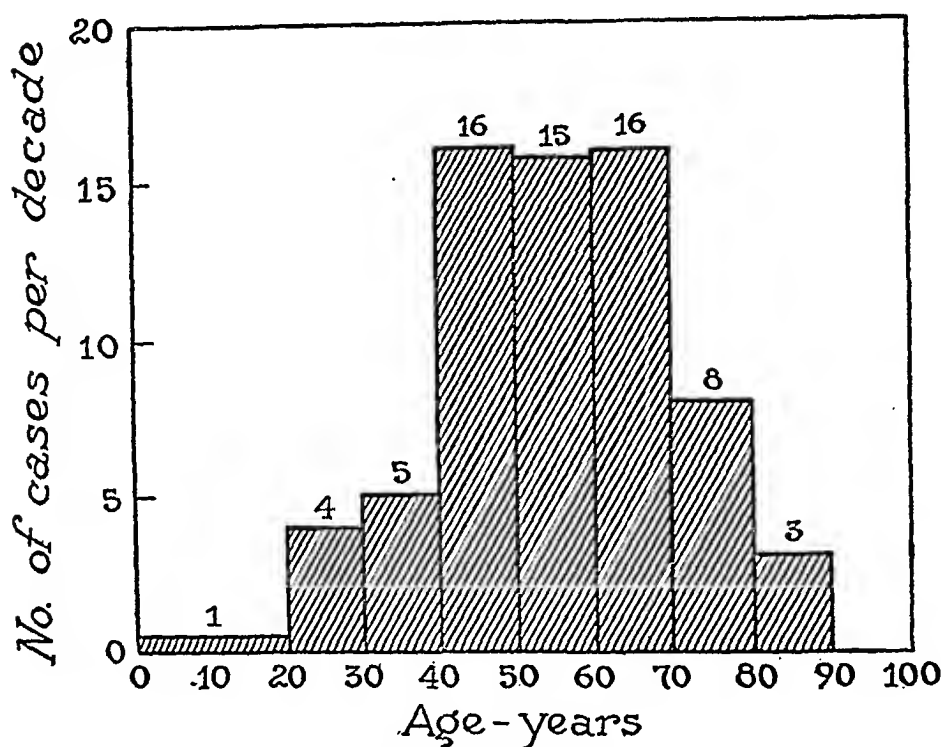
Ghon¹² also believed in the persistence of tuberculous infections. He stated that the primary complex may heal completely, but that in the majority of cases the healing is incomplete, the process passing from a state of activity to one of latency where it may give rise to endogenous reinfection. In discussing the latency of tuberculous infections Calmette¹³ mentioned that lymph nodes, especially in the peribronchial and mediastinal regions of adults, may contain small sclerotic foci that are frequently visible only microscopically. He stated that the majority of such lesions still contain virulent organisms of *Mycobacterium tuberculosis* which can be demonstrated by the inoculation of animals.

The literature reviewed therefore indicates that there is no unanimity of opinion concerning the infectivity of chronic lesions of the primary complex of tuberculosis. It is apparent, however, that morphological evidence is not a trustworthy criterion unless the findings are supplemented by the results of animal inoculation tests. Even then there may be difficulty in correlating the pathological changes as viewed microscopically with the results of the tests for virulence, since the identical tissue cannot be utilized for both procedures.

MATERIAL AND METHODS

In contemplating the study which forms the basis of this report, the possibility that lesions of long duration might contain viable

but attenuated or avirulent organisms of *Mycobacterium tuberculosis* was considered. In order to demonstrate acid-fast forms, which might be present but which might not induce demonstrable lesions in guinea pigs, it seemed desirable to inoculate culture mediums with portions of each specimen studied. In other words, an attempt was to be made to determine whether or not living



TEXT-FIG. 1. Age distribution by decades of the 68 human subjects who had chronic primary tuberculosis.

organisms of *Mycobacterium tuberculosis* were present by procedures that had proved dependable in the study of tuberculous materials from many sources.

Material was obtained at autopsy from a total of 68 unembalmed bodies of human beings who had died from causes other than tuberculosis. Evidence of infection with *Mycobacterium tuberculosis* as observed grossly was limited in most instances to well encapsulated caseous or caseocalcareous Ghon tubercles or similarly affected tracheobronchial lymph nodes. The ages of the respective individuals ranged from 7 to 90 years, the largest number having been in the fourth, fifth and sixth decades (Text-Fig. 1).

Twenty-two of the subjects were females, 46 males. There was only 1 negro in the series. The causes of death were as follows: malignant neoplasm, 26 cases; bacterial infection, 15 cases; cardiovascular disease, 7 cases; peptic ulcer, 4 cases; blood dyscrasia, 4 cases; uremia, 3 cases; pulmonary embolism, 3 cases; and miscellaneous causes, 6 cases.

The material obtained for subsequent studies to determine the presence or absence of organisms of *Mycobacterium tuberculosis* consisted of Ghon tubercles, hilar lymph nodes, or both Ghon tubercles and hilar lymph nodes from the same subject. In 5 cases tissues representing so-called apical scars were studied. In these instances, however, a Ghon tubercle or an involved hilar lymph node was also obtained for study. The tuberculous thymus gland was studied in 1 case and in another a tuberculous mesenteric lymph node was utilized. In both cases, however, the extrapulmonary lesions were studied in addition to the lesions that were present in the lungs. In a few cases 2 hilar lymph nodes or 2 Ghon tubercles from the same subject were used. A total of 103 emulsions was prepared from the material secured from these 68 subjects. These were as follows: from Ghon tubercles, 41; from hilar lymph nodes, 55; and from apical scars, thymus and mesenteric lymph node, 7.

Lesions that appeared macroscopically to represent a tuberculous infection were placed in sterile containers immediately after their removal from the body and delivered at once to the laboratory. In most instances a small portion of the lesion was preserved for subsequent histological study. As received at the laboratory the specimens consisted, for the most part, of grossly morbid tissue with a minimal amount of apparently normal tissue at the periphery. In a few instances in which generous amounts of pulmonary tissue containing Ghon tubercles were removed from the body, the lesions were separated from the surrounding tissues of the lung before the nodules of morbid tissue were emulsified. The attempt in every instance was to use for subsequent study only those tissues that constituted the lesion of tuberculosis as the disease is recognized at autopsy, and to avoid if possible the use of surrounding tissues which, while apparently non-tuberculous, might conceivably contain the infective agent in a state of latency.

The respective specimens were prepared for injection into ani-

mals and for the making of cultures by grinding them thoroughly with sterile sand in a small amount of physiological sodium chloride solution. The amount of fluid added to facilitate emulsification of the specimens seldom exceeded 6 to 7 cc. After being emulsified the material was transferred to a test tube which was placed in the refrigerator for from 24 to 48 hours. The supernatant fluid was then used for the making of cultures and for the injection of animals.

Cultures: From the emulsion prepared from each lesion two 1 cc. amounts were used for the making of cultures. For the purpose of eliminating other than organisms of *Mycobacterium tuberculosis* from the material to be cultured the oxalic acid method of Corper and Uyei¹⁴ was followed.* Because of the possible rôle of the bovine type of the tubercle bacillus in infections in human beings and because most strains of the bovine tubercle bacillus when first isolated are non-glycerophilic, it appeared desirable to provide a non-glycerinated as well as a glycerinated medium for the making of cultures. The medium selected was the unheated egg yolk-agar combination described by Herrold.¹⁵ † This was prepared in two forms: one in which glycerin to the amount of 3 per cent was incorporated and one to which no glycerin was added.

From each lesion that was emulsified 2 cc. of the fluid material previously treated with oxalic acid were distributed over the surface of 8 slants of medium, 4 being glycerinated and 4 containing no glycerin. The cultures were incubated at 37.5° C. for from 10 to 12 weeks before the final results were recorded.

Inoculation of Animals: Two guinea pigs were given injections of material from each lesion. Although in the majority of cases only 1 lesion was obtained, in some instances 2 or even 3 separate lesions were used. Consequently in a considerable number of cases 4 or even 6 guinea pigs received injections of the material. The total number of guinea pigs used was 206. All injections were made subcutaneously into the tissues of the wall of the belly.

The dose varied and depended on the amount of material avail-

* The method consists of adding an equal amount of 5 per cent oxalic acid solution to the material to be cultured. The mixture is then placed in the incubator at 37° C. for 30 minutes. It is then diluted with 5 volumes of physiological sodium chloride solution and centrifuged for 30 minutes. The supernatant fluid is then discarded and the precipitate spread over the surface of the culture medium.

† In preparing this medium 15 gm. of agar per liter was used instead of 10 gm. as originally proposed by Herrold.

able. At least 1 cc. was injected into each guinea pig and in many instances 2 cc. or more were given. When guinea pigs died within 21 days after having been given the injections the results were considered as failures. The guinea pigs were killed after the lapse of 8 weeks or longer unless they had died before that time. A large number were permitted to live for 12 or even 14 weeks before being killed for autopsy. The results for 36 guinea pigs that died within 3 weeks after the injection were recorded as failures. In only 3 cases, represented by 8 guinea pigs, did all of the animals die within 3 weeks. The other 28 guinea pigs that died within 3 weeks had been given injections of material from 22 subjects. However, other guinea pigs had been inoculated with portions of the same material from these 22 subjects, and these guinea pigs lived more than 21 days. In the latter instance the survival period was sufficient for lesions of tuberculosis to become evident if the material injected had been infective. Consequently it was possible to draw conclusions as to the infectivity of the material for guinea pigs in all but 3 cases.

The guinea pigs were examined carefully at autopsy and all lesions, even though but slightly suggestive of tuberculous infection, were removed for additional study to determine whether or not *Mycobacterium tuberculosis* was present. The procedure consisted of the making of cultures, the inoculation of additional guinea pigs and the examination of portions of the tissues histologically.

RESULTS OF LABORATORY EXAMINATIONS

Positive results were obtained for only 1 of the 68 subjects. In none of the others was the presence of *Mycobacterium tuberculosis* demonstrable by cultural methods or by animal inoculation.

This single subject for whom positive results were obtained was a 54 year old male who had died as a consequence of fracture of the skull. The material obtained for tests for infectivity consisted of 1 encapsulated caseocalcareous Ghon tubercle from each lung. The respective lesions were emulsified separately and each emulsion was used to prepare cultures and to inject guinea pigs. There was no evidence of the presence of viable organisms of *Mycobacterium tuberculosis* in the emulsions made from the lesion of the left lung. From the lesion from the right lung, however, colonies

of acid-fast bacilli were observed after an incubation period of 52 days and lesions of tuberculosis were present at autopsy in both of the guinea pigs that had been given injections of portions of the same emulsion used to make the cultures. One of these guinea pigs died after 32 days. There was an ulcerated area in the skin where the material had been injected. The spleen was slightly swollen and, macroscopically, contained indistinct foci suggestive of a tuberculous infection. Histologically the spleen showed the presence of numerous tubercles in which, by appropriate staining methods, acid-fast bacilli were demonstrated. The other guinea pig died 55 days after having received the inoculum and was found at autopsy to be affected by an extensive tuberculous process. Subsequent studies indicated quite definitely that the organism of *Mycobacterium tuberculosis* demonstrated in the Ghon tubercle from the right lung was of the human type.

HISTOPATHOLOGICAL CHARACTERISTICS OF THE LESIONS

In 41 cases histological sections from 46 tuberculous foci were made and stained with hematoxylin and eosin. In some cases decalcification of the material was necessary, but otherwise the routine technique was used. In 27 cases sections were not obtained because the tuberculous foci were so small that the entire lesion was used for the injection of guinea pigs and for the inoculation of culture mediums. The lesions were classified into three groups on the basis of their histological appearance. In the first group were placed those lesions that revealed no evidence of activity whatsoever; in the second, those that showed slight or only minimal evidence of activity; and in the third, those lesions that appeared definitely active histologically.

The first group consisted of lesions from 20 subjects, an example of the type of lesion characteristic for this group being shown in Figure 1. It is apparent from this figure that the lesions in this group consisted essentially of a caseous or calcified central area of varying size surrounded by a zone of dense fibrous, and often hyalinized, connective tissue. Giant cells, epithelioid cells, or actively proliferating fibroblasts were not seen in these lesions. An interesting finding in this group was the presence of bone in 5 lesions. In 3 of these the formation of marrow had actually occurred.

The second group of lesions, which were designated as showing slight evidence of activity, was from 22 subjects. Microphotographs of sections from 2 of these lesions from the same subject are shown in Figures 2 and 3. The centers of these lesions, like those of the first group, were always caseous or calcified or both, but the periphery showed, in addition to dense fibrous connective tissue, areas or isolated foci of fibroblasts with or without giant cells. Some of the giant cells were of the Langhans type whereas others were morphologically atypical. In regard to this group it may well be argued that since the lesions were often calcified and always encapsulated by a zone of dense fibrous connective tissue, they should be considered inactive histologically, regardless of peripheral fibroblastic activity. Many workers would probably favor such an interpretation. As a working classification, however, we found this grouping useful even though it may be considered arbitrary.

The third group of lesions was from 4 subjects. These were lesions which we designated as revealing definite histological evidence of tuberculous activity. There was active proliferation of fibroblasts, usually with the association of typical giant cells at the periphery of a caseous area. In all instances there were secondary tubercles beyond the primary focus. These secondary foci had small necrotic centers with surrounding zones of epithelioid cells and lymphocytes. Typical giant cells of the Langhans type were frequent. An example of this type of lesion is shown in Figure 4.

In 5 cases sections of multiple tuberculous foci from the same subject fell into different groups. Thus there were 2 cases in which sections of the hilar nodes revealed inactive lesions, whereas sections from the pulmonary nodules appeared slightly active. In 2 other cases the pulmonary lesions were classified as inactive, whereas the hilar nodes showed slight evidence of activity. In 1 case sections of the hilar nodes revealed minimal evidence of activity, whereas sections of the thymus revealed an inactive lesion.

The lesion in the 1 case that was proved by cultures and by inoculation of guinea pigs to contain virulent tubercle bacilli was found to show but slight evidence of activity histologically and had been placed in the second group. The sections were re-examined carefully in order to determine whether or not they could

be placed in the group designated as active histologically. Careful search, however, convinced us that there was insufficient evidence of activity to place them in this group. A microphotograph of a section of a Ghon tubercle in this case is shown in Figure 5. There was a central area of caseation and a peripheral zone of dense hyalinized fibrous connective tissue. Cholesterol crystal clefts were present, both in the central caseous area and in the fibrous capsule. The continuity of this zone of hyalinized fibrous tissue was broken by foci of actively proliferating fibroblasts, lymphocytes and phagocytes laden with a brownish pigment.

Sections of the hilar node in this case were quite similar to those of the pulmonary nodule. There was a large central area of caseation which showed beginning calcification. There was a peripheral zone of dense hyalinized connective tissue broken here and there by small collections of lymphocytes and fibroblasts. No giant cells or secondary tubercles were present. A finely granular, brownish pigment was present in the fibrous tissue at the periphery of the lesion. Similar pigment also occurred in the phagocytic cells of the non-tuberculous portion of the lymph node. This portion of the section, as well as similar areas in the section of the pulmonary nodule, was found to contain large amounts of silica on examination with the polarizing microscope.

PRESENCE OF SILICA

On microscopic examination of the sections prepared from the tuberculous lesions we were impressed by the frequent occurrence at the periphery of a brownish granular pigmentation of the connective tissue. The pigment was often associated with dense whorls of collagenous connective tissue (Fig. 6). At other times the pigment was found in phagocytes. In some areas it had the appearance of hemosiderin and in others it was quite evidently carbon pigment. Because of the dense fibrosis associated with the pigment it was thought wise to investigate the silica content by means of the polarizing microscope. Silicious deposits were found in large amounts in all such areas.

In most instances the silica occurred chiefly at the periphery of the lesions. It was thought therefore that the presence of silicious deposits might explain why so many lesions showed histological evidence of slight activity although the presence of active tuber-

culous infection could not be demonstrated by culture or by inoculation of guinea pigs.

In order to establish this point all sections were examined under the polarizing microscope and graded as to silica content on the basis of 0 to 4. It was found that practically all the lesions contained some silica and that the deposits were chiefly at the periphery of the lesions in the fibrous tissue.* However, on comparing the silica content with the presence or absence of histological signs of activity, it was found that no correlation existed. That is, many histologically inactive lesions contained large amounts of silica at the periphery, whereas other lesions classified as active or slightly active contained minimal amounts of silica. None of 4 cases in which the lesions contained secondary tubercles had more than a Grade 1 or Grade 2 deposit of silica.

COMMENT

The results of this study lend support to the belief that, in the majority of instances, lesions of the primary complex of tuberculosis pass through an involutional process that is unfavorable to the continued viability of *Mycobacterium tuberculosis*. In further support of this interpretation is the fact that though the primary complex is present in 70 to 90 per cent of human beings of all age groups, only a relatively small percentage of the population manifests clinical signs of tuberculosis. Additional evidence for believing that the bacilli responsible for the primary complex of tuberculosis eventually become innocuous is the recent observation of Long and Siebert.¹⁶ They noted that a small number of subjects with calcified lesions of the childhood type of tuberculosis did not react to P.P.D. or to other forms of tuberculin. Similar findings had previously been reported by McPhedran and Opie.¹⁷ They found in a large series of subjects representing 1000 families that calcified tuberculous foci were present in approximately 5 per cent of the individuals who failed to react to the tuberculin test. Whether or not the sensitivity to tuberculin diminishes with advancing age in individuals whose tuberculous infection is limited to the primary complex provides an interesting question. It seems

* In none of these cases was there any doubt as to the original cause of the lesions, that is, they were all grossly and histologically of tuberculous origin. In none of these cases was there any evidence of generalized pulmonary silicosis.

possible, at least theoretically, that sensitivity to tuberculin should diminish as the bacteria responsible for its induction disappear or become non-viable. It has been established experimentally that dead tubercle bacilli may provoke a state of sensitivity to tuberculin. When the lesions are of long duration and contain relatively few bacilli that are non-viable, the ability of the tissues to react to tuberculin may no longer be evident.

The results of our study seem to support those of Griffith who concluded that the chronic lesions of tuberculosis undergo spontaneous arrest and that in such lesions living tubercle bacilli can rarely be demonstrated. Griffith's results as well as our own are definitely at variance with the findings of several other investigators, especially those of Opie and Aronson. The latter obtained positive results by the inoculation of guinea pigs in 30 per cent of the material they examined. However, they did not maintain that the infective agent was present within the substance of the lesions, but rather that it was derived, with few exceptions, from the surrounding lung or lymphoid tissues.

In the material utilized in our work an attempt was made to include in the inoculum only the substances constituting the morbid process. However, the nature of the material precluded the possibility of attaining this objective in all instances. While no considerable amount of the adjacent pulmonary or lymphoid tissue was included in the respective emulsions, at least a small fringe of the surrounding tissues was in many instances incorporated with the morbid tissues from which the inoculums were prepared. While Opie and Aronson's findings possibly provide an explanation for the one positive result we obtained, nevertheless the fact that a small amount of the surrounding, apparently non-tuberculous tissues was included in many of the emulsions we used makes this interpretation unsatisfactory for explaining the negative results in all of the other cases. The only interpretation that seems tenable is that the tissues which failed to produce tuberculosis in guinea pigs or to yield positive cultures of *Mycobacterium tuberculosis*, in our experiment, were devoid of living or virulent forms of this bacterium.

The methods used to demonstrate the tubercle bacillus, if it had been present in a viable virulent state, were those in which reliability has been amply established. To have used cultural pro-

cedures alone would have justified a criticism concerning the adequacy of the methods. However, since for every specimen 2 guinea pigs were used, in addition to 8 tubes of culture mediums, it would seem that had living tubercle bacilli been present they should have become manifest. Their failure to do so properly justifies the conclusion that the material examined did not contain living *Mycobacterium tuberculosis*.

In the attempt to correlate the histopathological findings with the results obtained by the injection of guinea pigs and the inoculation of culture mediums, we were constantly aware of the fact that the material used for the histological study could not be identical with that used for the laboratory tests. As a rule the lesions were cut approximately in half and there was no possible way of ascertaining that the portion used for histological study was the same, either as to morphological characteristics or bacterial content, as the portion used for the injection of guinea pigs and for the making of cultures. Consequently, there is the possibility that in the 4 cases that showed lesions which were active histologically the portion of the nodule from which sections were prepared may have contained viable organisms of *Mycobacterium tuberculosis*, whereas the portion used for the inoculation of culture mediums and for the injection of guinea pigs did not contain the bacilli. Had this latter portion been examined there is the possibility that it would not have appeared active histologically.

Although these possibilities exist they do not constitute a satisfying explanation for the lack of correlation. A hypothesis which may explain the apparent discrepancy between histological evidence of activity as seen in the lesions of the third group and the negative results from the laboratory tests has been advanced by Beitzke.¹⁸ He believed that the secondary, apparently active tubercles at the borders of encapsulated and calcified tuberculous foci are not produced by tubercle bacilli which escape from the primary lesion, but are an allergic response to fresh infection with the infective agent. According to Beitzke, the phenomenon is similar to that which takes place when tuberculin is injected into sensitized animals and sufficient antigen is present in the tissues to provoke a reaction.

Finally, the possible significance of silica remains to be considered in the interpretation of the results. Although no correla-

tion existed between the quantity of silica present and the extent of histological signs of activity, it is nevertheless felt that in many of the lesions which showed histological evidence of activity the causative factor was silica rather than viable tubercle bacilli. This, we believe, is especially true of those lesions that showed minimal evidence of activity, that is, the well encapsulated caseous or calcified nodules of the second group which revealed only slight fibroblastic activity at the periphery and no typical tubercles.

SUMMARY AND CONCLUSIONS

A study was made to determine the presence of virulent tubercle bacilli in chronic tuberculous lesions of the lungs and contiguous lymph nodes of human beings who had died of causes other than tuberculosis. The lesions were emulsified and material from the respective subjects was used to inoculate culture mediums and to inject guinea pigs. Material from a total of 68 subjects was utilized and negative results were obtained in all but 1 case. The age distribution of the individuals studied ranged from 7 to 90 years, approximately 70 per cent having been in the fourth, fifth and sixth decades of life. Tissues from the lesions of the majority of the subjects were studied microscopically and evidence of activity was apparent in a considerable number. The presence of silica was demonstrated within most of the lesions. The results of this study seem to justify the following conclusions:

1. The lesions of the primary complex of tuberculosis, when definitely encapsulated and sclerotic, or caseous or caseocalcerous, seldom contained viable or virulent organisms of *Mycobacterium tuberculosis*.
2. The presence or absence of viable or virulent organisms of *Mycobacterium tuberculosis* in the lesions of the primary complex of tuberculosis cannot be established by morphological appearances alone.
3. The data suggest that in adults endogenous reinfection is unlikely to occur from lesions of the primary complex.
4. The presence of silica in varying amounts is a fairly constant finding in lesions of the primary complex of tuberculosis. In the absence of demonstrable viable tubercle bacilli in the lesions it is suggested that histological signs of activity are possibly due to silica.

NOTE: We wish to acknowledge the assistance of Drs. Lall G. Montgomery, D. C. Beaver and J. C. Henthorne, who aided materially in collecting the specimens utilized in this study.

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DESCRIPTION OF PLATES

PLATE 127

- FIG. 1. Ghon tubercle with no histological evidence of activity. $\times 85$.
- FIG. 2. Portion of periphery of a Ghon tubercle showing fibroblasts, mononuclear cells and lymphocytes which suggest activity of a mild degree. $\times 130$.
- FIG. 3. Portion of a hilar lymph node from the same subject. One giant cell of the Langhans' type is present in the midst of hyalinized connective tissue. $\times 130$.

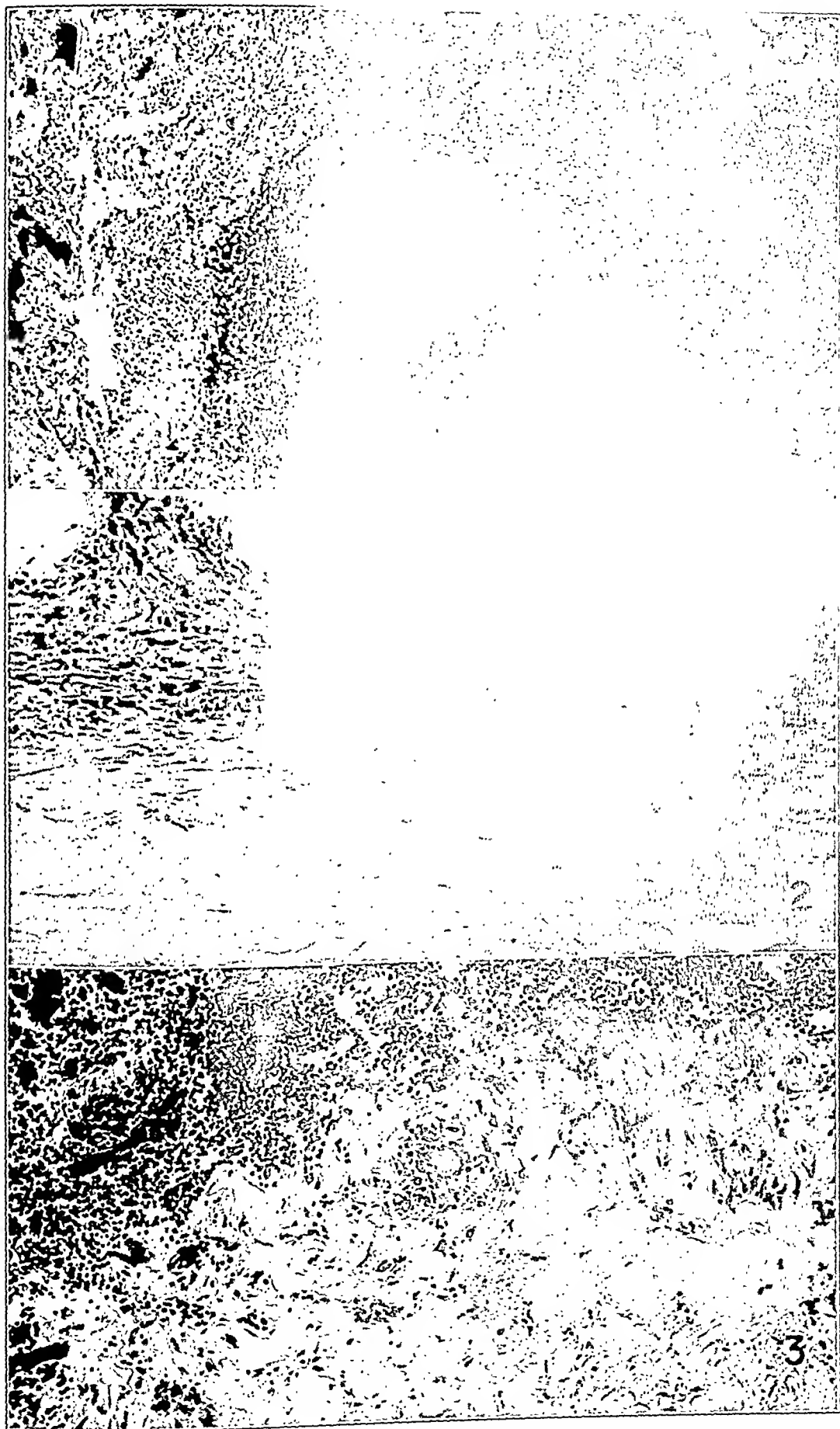
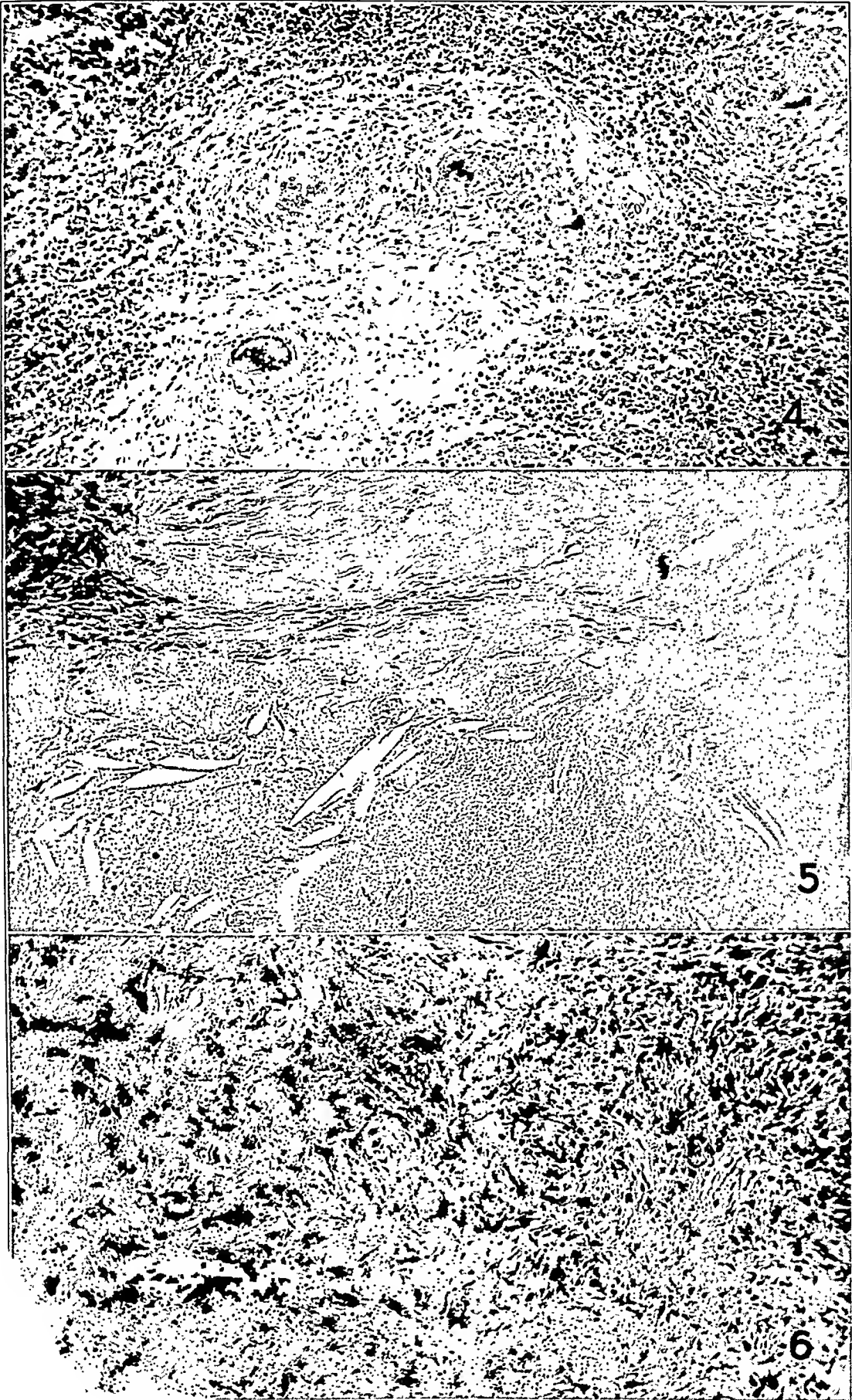


PLATE 128

- FIG. 4. Conglomerate tubercle with several giant cells in a hilar lymph node. $\times 100$.
- FIG. 5. Portion of Ghon tubercle in which virulent tubercle bacilli were demonstrated. $\times 130$.
- FIG. 6. Hilar lymph node showing evidence of activity of the connective tissue elements. Large amounts of silica were observed in this lesion by polarized light. $\times 120$.



THE STAINING OF ACID-FAST BACILLI IN PARAFFIN SECTIONS *

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GENERAL CONSIDERATIONS

Ficker ¹ in 1929 reviewed and collected from the literature 95 articles dealing with methods of staining acid-fast bacilli, chiefly in smears of cultures and sputums, parenthetically stating that the best review of the literature listed 716 articles on the subject. The number of methods directed at the staining of bacilli in sections is much smaller than this, but still so large that it would be nearly impossible to undertake to examine all the variations critically by comparing them. It would seem probable that there is no single method of staining acid-fast bacilli in tissues capable of meeting all demands and individual tastes, and it is our purpose here not to offer a method calculated to satisfy all difficulties but to consider the various factors involved and the difficulties that the problem presents. No effort is made to review the literature on the subject adequately, or to claim originality for many of the observations made. It would be difficult to introduce a chemical or dye substance into the staining process which has not long since been tried and found useful or discarded.

Koch, ² in his original paper (1882) on the discovery of the tubercle bacillus, described the staining of bacilli in tissues with alkaline methylene blue, counterstained with vesuvin (Bismarck brown). Ehrlich and he in the same year employed an aniline water methyl violet or fuchsin; Ziehl ³ substituted phenol for the aniline and Neelsen in 1885 devised the general formula for carbol fuchsin that is largely used today. Much ⁴ in 1907, by modifications of Gram's method (1884), demonstrated granular forms not stained by the Ziehl method. In the innumerable subsequent methods only the dyes of the triphenylmethane group have been much used, pararosaniline and its derivatives. The greater bril-

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liancy of the bacilli stained with fuchsin has apparently proved on the whole superior to the darker color of methyl and crystal violet, judging from the greater popularity of the former.

FIXATIVES

The original observation that *alcohol is the fixative of first choice* for purposes of staining acid-fast bacilli in sections has not been located, but the many authors who have compared the virtues of different fixatives invariably agree upon it. The fact cannot be exaggerated; in tissues fixed in alcohol acid-fast bacilli are stained rapidly and resistantly. The importance of alcohol fixation is much greater when one is dealing with acid-fast organisms difficult to stain in tissues, such as the human lepra bacillus. Alcohol is not, however, a generally satisfactory tissue fixative; it produces much shrinkage around the margins of the piece of tissue. With larger fragments this is not of as great a disadvantage as when quite small pieces of tissue are to be sectioned. The addition of 10 per cent formaldehyde to the alcohol improves the fixation without affecting the staining of the bacilli. Klingmüller⁵ recommends the use of 5 per cent mercuric chloride in alcohol as a fixative. The use of increasing strengths of alcohol avoids part but not all of the shrinkage. In ordinary pathological work the foresighted use of alcohol as a fixative is a most uncommon practice, but in experimental work the disadvantages of alcohol will often be found overshadowed by its virtues.

The various fixing fluids containing mixtures of potassium dichromate and formaldehyde are combinations of an oxidizing and a reducing agent, which react to oxidize formaldehyde and to reduce potassium dichromate with the deposition of chromium salts in the tissues. Joannović⁶ states that formaldehyde alone is a poor fixative for acid-fast bacilli, though others disagree. It is probable that formaldehyde is fairly effective in fixing the lipoids of the acid-fast bacilli. Our experiences with decalcifying fluids (such as 5 per cent nitric acid) indicate that these are most deleterious to the achievement of acid-fast stains. This suggests that the formic acid formed in Orth's or Zenker's fluid with formaldehyde might exert a harmful effect. Actually the formic acid formed in these fluids is probably largely decomposed. If tissues are left standing in formaldehyde alone, however, much formic acid is formed, and

tissues preserved in formaldehyde for long periods are nearly useless for acid-fast stains. On the other hand, it seems that *brief* fixation in neutral formaldehyde, while not ideal, may be satisfactory.

That chromium salts are deposited in the lipoids of acid-fast bacilli can be directly observed by adding a solution of potassium dichromate to a culture of tubercle bacilli. This does not affect their tinctorial properties, nor is their acid-fastness lessened by soaking in alcohol and chloroform. But when the culture, treated 30 minutes with potassium dichromate, dehydrated in alcohol and chloroform, is treated with melted paraffin at 55–60° C., the acid-fast properties of the bacilli begin to weaken. There is much confusion concerning the exact effects of more prolonged treatment of sections in chloroform, xylol and paraffin, but it is quite clear that ill effects are particularly observed in tissues fixed in potassium dichromate. Joannović and Klingmüller both emphasize the fact that the disadvantages of fixation in Orth's, Müller's, or Zenker's fluid can largely or completely be overcome by thoroughly washing the excess fixing fluids out of the tissue in running water. This is undoubtedly of the greatest importance, but it is probably not true that tissues so treated ever measure up to tissues fixed in alcohol, for purposes of staining acid-fast bacilli. We have never used Zenker's fluid containing acetic acid, but it appears that the mercuric chloride in the solution does not affect the staining of acid-fast bacilli, although its action as a general fixative is useful.

The deleterious effect of autolysis on acid-fast bacilli is probably measurable. In thick blocks of tissue in which the central part is not fixed, or poorly fixed, the difference in the tinctorial properties of bacilli in the fixed and unfixed parts of the block is noticeable. Successful staining of acid-fast bacilli in sections demands careful attention to the process of fixation.

CLEARING OF MERCURY DEPOSITS FROM TISSUES

The classical method of removing the mercury deposits from the tissues with Lugol's iodine-potassium iodide mixture is wholly satisfactory, but, unless completely removed, traces remaining are likely to cause unsightly insoluble precipitates of fuchsin in the section in their neighborhood. Any acid-fast organisms nearby such deposits will be most brilliantly stained. Removal of the

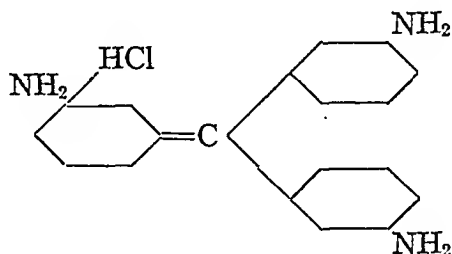
iodine by alcohol is often a slow process; conversion by sodium thiosulphate into sodium iodide is very rapid, but tends to leave water-insoluble deposits which will precipitate fuchsin. Traces of iodine left in the section may not interfere with the staining process, but it seems best to treat the section with both alcohol and sodium thiosulphate (in this order) to avoid all difficulties.

OXIDATION AND REDUCTION

It has been our experience, especially with tissues fixed in fluids containing potassium dichromate, that treatment with potassium permanganate 0.25–1 per cent for a few minutes, followed by oxalic acid, definitely improves the staining of acid-fast bacilli, although it will by no means obviate the disadvantages of these fluids. This process tends to loosen sections from the slides and to cause shrinkage. In some instances overtreatment may loosen lepra bacilli from the cells, extracting them from the section. Gömöri ⁷ advises the use of a gelatin-glycerin mixture for affixing sections to slides, followed by drying in formaldehyde fumes. We have, however, encountered no difficulties if a glycerin-albumin mixture is used, prepared from the whites of fresh eggs.

FUCHSIN

Basic fuchsin, magenta and aniline red are names under which mixtures of pararosaniline and its three methylated compounds are sold; most commonly it is a mixture of pararosaniline and rosaniline.



The above formula is that usually given for pararosaniline chloride. It has an unsaturated carbon atom in each phenyl group, and one to three methyl groups may be substituted at these points, yielding four possible compounds. In practice the methylated dyes are prepared by oxidizing equivalent mixtures of aniline and toluidine, with the aid of a metallic catalyst, and are purified by treating with an acid, yielding salts of rosaniline and its derivatives.

Pararosaniline, rosaniline (with one methyl group), and the trimethylated compound (new fuchsin) are obtainable in pure form.

By treating pararosaniline with methyl or ethyl iodide, methyl or ethyl groups replace the hydrogen atoms in the amine groups, anywhere from three to six in number. The lower triethylated compound largely composes the dye known as dahlia or Hoffmann's violet. Mixtures of the methylated compounds are known as methyl violet. The pure hexamethyl pararosaniline is crystal violet.

In our experience the trimethylated pararosaniline (with the methyl group attached directly to the phenol ring), triamino-tritoly methane chloride, fuchsin NB, magenta III, isorubin, or, most commonly, *new fuchsin*, possesses much greater powers of staining acid-fast bacilli than do the pure simpler compounds, or any of a number of samples of basic fuchsin we have employed. It possesses a somewhat darker, less red and more magenta tint than does rosaniline, but the dyed bacilli display greater sharpness of outline and deeper staining. Irrespective of the fixative employed, when adjacent sections are stained for varying periods of time for purposes of comparison, in new fuchsin and rosaniline, it has always been observed that the new fuchsin acts more rapidly, giving a more uniform result and deeper stain. It is only when the time of staining (at room temperature) is prolonged that the effect of rosaniline approaches that of new fuchsin. It seems that new fuchsin should wholly displace the other fuchsins as a dye for staining acid-fast bacilli in tissues.

CARBOL FUCHSIN

1. Acid-fast bacilli can be effectively stained in a simple aqueous solution of fuchsin at room temperature under favorable conditions of fixation; many hours may be required.

2. A saturated solution of fuchsin in 95 per cent alcohol leaves the bacilli neither acid- nor alcohol-fast after several days' staining.

3. Acid-fastness and alcohol-fastness are wholly relative terms. The best stained acid-fast bacilli are eventually wholly decolorized by alcohol (2-3 days). Strong acids alone accomplish the same effect, possibly with eventual decomposition of the fuchsin, but in a wholly different manner.

4. The acid-fast staining reaction is thus a reversible one. It

takes place poorly or not at all in solutions of fuchsin in solvents in which the fuchsin is readily soluble. Saturated solutions of fuchsin in aniline or phenol, having a dye content many times that of Ziehl-Neelsen's carbol fuchsin, produce indifferent results.

5. The following three solutions have been compared:

A. New fuchsin	0.25 gm.
Phenol crystals	5 gm.
Water, to make	100 cc.
B. New fuchsin	0.25 gm.
Alcohol	10 cc.
Water, to make	100 cc.
C. New fuchsin	0.25 gm.
Phenol crystals	5 gm.
Alcohol	10 cc.
Water, to make	100 cc.

Only the third solution (C) is a highly effective staining solution. Both the 10 per cent alcohol and the 5 per cent phenol are essential.

6. If the section is treated before staining in:

Phenol crystals	5 gm.
Alcohol	10 cc.
Water, to make	100 cc.

the staining takes place much more rapidly. Omission of either ingredient from the solution largely nullifies the effect. The alcohol is as important as the phenol for the enhancement of the staining; its rôle is more than that of a solvent for the fuchsin.

7. Many substances have been substituted for the aniline or phenol in the preparation of fuchsin, or added to the mixture — boric acid, petroleum ether, aniline black,^{8, 9, 10} glycerin,¹¹ and salt.¹² We have ourselves tried in addition many other compounds, phenols, amines, salts, and so on. Nothing has been found giving results superior to phenol; many substances, however, are more or less effective, particularly when they are present in concentrations close to the point at which fuchsin is precipitated by them.

8. The phenol in carbol fuchsin apparently plays a complicated rôle; it appears to combine with the fuchsin and with the acid-fast material in the bacilli. Its rôle in dissolving the fuchsin is less certain.

USE OF THE DYE

One of the most variable factors in all modifications of methods employing fuchsin has been the length of time and amount of heat required to stain the bacilli. There are three common methods employed: (1) staining at room temperature; (2) staining immersed in the dye at elevated temperatures, 37–70° C.; and (3) staining by steaming the fuchsin on the slide. Each may be said to have its virtues. The first is the simplest and slowest. The second is the most effective and most injurious to the tissues. The great virtue of the third is its rapidity. For comparing the effectiveness of different dyestuffs or solutions we have used only the first.

Time and heat play the same rôle in the mechanism of the acid-fast reaction. Results are obtainable by staining at room temperature which are wholly the equal of those obtained by using heat. The lower the temperature, the longer will be the time required. The length of time, or the amount of heat required, is also dependent on the method of fixation of the tissue. Steaming the slide is probably the least reliable method in unpracticed hands; it is inconvenient for staining large numbers of sections at one time.

It may seem an unnecessary tautologism to emphasize that the important point of any method is to obtain adequate staining of the organism. The process should be carried to the point beyond which there is no further accumulation of the dye in the bacilli. Much examination of incompletely stained bacilli leads us to believe that the staining takes place in a peculiar but orderly fashion, because the material in the organism giving the acid-fast reaction is by no means evenly distributed through the bacilli, but is concentrated at a few places. Incompletely stained bacilli are usually not weakly and evenly stained but often have a beaded or granular appearance, even to appearing as masses of cocci. With more intensive staining they appear as solid, evenly stained shafts. When acid-fast bacilli occurring in numbers show this granular effect it is almost certain that the tinctorial effect is not maximal. We have no doubt that much of the effect of Much's staining methods is due to their inadequacy completely to stain the acid-fast matter in the bacilli, paradoxically combined with the superiority of crystal violet to most basic fuchsins in staining acid-fast organisms.¹³

The commonly seen beaded appearance has often been considered possibly to be somewhat of an artefact. Cowdry and Heimburger¹⁴ reported that by using Gersh's technique of freezing the tissue in liquid air and dehydrating the solidified tissue *in vacuo*, this beaded appearance was not seen when the tissues were stained with fuchsin.

What has been said above of heat concerns its effect on the staining process, that is, the chemical reaction. But there is a separate effect of heat involved necessarily in staining with the aid of heat, namely, the effect on the carbol fuchsin itself. This results in the greater and more brilliant redness acid-fast substances display when stained by fuchsin which has previously been heated to the boiling point. The greater brilliance is obtained equally well when the bacilli are stained cold with a previously heated solution, as when the bacilli are stained by an ordinary solution with the aid of heat. On cooling a heated carbol fuchsin a good deal of the dye is precipitated, which vitiates the strength of the solution. Many writers caution against the use of heat in preparing the solution.

Thus, heating, which produces greater brilliance and more rapid staining, is a mixed blessing. The disadvantages are waste of dye, greater injury to sections, and a distinct tendency to alter the form and outline of the bacilli, making them appear shorter and plumper, or, in leprosy, causing the masses of bacilli (*globi*) to fuse together and lose individual distinctness.

The amount of fuchsin called for by different methods varies from 0.3–1 gm. per 100 cc. We have seen very few samples of basic fuchsin that are sufficiently soluble to yield the latter strength. Conn¹⁵ gives the solubility of new fuchsin in alcohol as 3.2 per cent and in water as 1.13 per cent, but we have not seen samples possessing any such great water solubility as this.

When solutions of carbol fuchsin are prepared from a saturated alcoholic solution and 5 per cent phenol water, the resulting strength will be only that of 1/10 the solubility of the dye in alcohol. With pararosaniline this would be theoretically 0.59 per cent, with rosaniline 0.82 per cent, and with new fuchsin 0.32 per cent. Grüber's "fuchsin for bacilli" is the most soluble basic fuchsin we have seen, 1 per cent carbolated solutions being preparable from it, but the dye content appears to be well below that of a pure

rosaniline. Few of the American products yield more than a 0.3 per cent solution, but they appear to be as effective as Grüber's.

Fuchsin in carbolated solutions slowly enters into a partly colloidal state (especially if tap water is used) which probably lowers its effective strength. Manifestly, the effectiveness of any solution is directly proportionate to the dye content, but the use of saturated solutions predisposes to precipitation. A saturated carbol new fuchsin contains approximately 0.9 per cent dye. New fuchsin is somewhat more soluble in methyl than ethyl alcohol, and we have used the following solution which has an effective dye content of 1 per cent:

New fuchsin	1 gm.
Phenol crystals	5 gm.
Methyl alcohol	10 cc.
Distilled water, to make	100 cc.

The dye is wholly dissolved in the mixture of alcohol and phenol, and the distilled water is added, not too rapidly. The solution should be clear and not require filtration. Fuchsin is so readily precipitated by a wide variety of substances that to maintain the full strength distilled water of high purity must be used. The solution preserves indefinitely, but it should be remembered that evaporation of 5 per cent by volume, which will largely be alcohol, will precipitate dye to a much greater proportion. Solutions left in Coplin jars lose strength rapidly.

DECOLORIZATION AND DIFFERENTIATION

The second variable in many modifications of acid-fast stains lies in the manner of decolorization. Alcohol is an essential ingredient, but the number of acids that has been suggested is very large.

There is probably nothing more futile than trying to accomplish decolorization of the tissues without that of the bacilli by employing weak acids or weak solutions of strong acids when the bacilli are not too well stained. It can be done at times but not with consistency. Not all mineral acids exhibit the same power of extracting the fuchsin. Hydrochloric acid is most effective, nitric acid less so, and sulphuric still less; but there is no acid highly ionized which is wholly ineffective and there is really not much choice be-

tween several. Fuchsin undergoes a change of color in acid from red to yellow but combined with tissues it may be blackened. With sulphuric acid some of the dye is sulphonated to form acid fuchsin so that the solution regains the red color. In alcoholic solution the color change is different. In weak acid alcohol the fuchsin becomes first a darker bluish red, gradually turning to a deep bluish violet and only turning yellow when the proportion of acid is much higher than ordinarily used. An acid alcohol which turns the dissolved fuchsin yellow also decolorizes the bacilli rapidly.

The classical 1-5 per cent hydrochloric acid in alcohol (70-95 per cent) is wholly satisfactory. The higher percentage of acid leaves the acid alcohol blue, the weaker leaves it red. The lower strength has nearly the same decolorizing effect as the higher, and is preferred. When the acid alcohol leaves an undesirable amount of fuchsin in the sections it can rarely be further decolorized by a stronger acid.

There are various methods that use the acid and alcohol separately. Much stronger acids are employed. The same strengths in alcohol would decolorize the bacilli. In other words, the acid is much more effective in alcoholic than in aqueous solution. The problem in staining acid-fast bacilli in sections rarely consists of a test of the acid-resistant properties of the organism, and such procedures seem to possess no advantages.

The use of picric acid has been made the *punctum saliens* of many methods and is an alluring medium, as we have found. Perhaps the picric acid, which may be effectively substituted for iodine in Gram's stain, behaves in the same way toward fuchsin. But it is not to be recommended for sections, for, unless the picric acid is removed by acid alcohol, the bacilli fade.

The pale pink residual background (sometimes fairly deep) may be treated in three ways: (1) it may be allowed to remain; (2) it may be masked by a heavier diffuse blue counterstain; and (3) it may be removed. The amount of residual fuchsin in the section depends on the time and heat used in the staining process, being greater with prolonged staining and heaviest in tissues subjected to long periods of fixation in heavy metals. In tissues fixed in formaldehyde the red blood corpuscles retain much dye. It cannot be removed by acids or alkalis. The difficulty with obliterating a heavy residue by a heavy counterstain (Delaney¹⁶) lies in

the fact that the heavy counterstain necessitates a very brilliant lighting, and the bacilli may take some of the counterstain. Under some circumstances this may be necessary.

When the residual color is quite light it may form a fairly satisfactory background of itself, not in any way confusing the brilliant color of the bacilli. In some tissues, such as the skin, the effect is often excellent when a simple nuclear counterstain, such as hematoxylin, is employed.

The removal of the residual fuchsin can be accomplished in several ways: by bleaching with potassium permanganate and oxalic acid, or by treatment with any of several substances which decolorize fuchsin. The coloring of the bacilli is not wholly resistant to any of these procedures, but brief decolorization will often serve to remove much of the residual dye from the tissues without seriously affecting the color of the bacilli. We have used with success a 5 per cent aqueous solution of potassium cyanide. Other substances, such as sodium metabisulphite, trichloroacetaldehyde, aqueous benzaldehyde, aniline and sodium sulphite, have been recommended. The difficulty with the sulphites, and other substances which decolorize fuchsin rapidly, lies in the fact that the reaction of decolorization is reversible. Extraction of the dye from the tissues is slower and is inevitably accompanied by the removal of some from the bacilli. Such procedures must be used with caution, but they are at times very helpful.

THE COUNTERSTAIN

The choice of the counterstain permits of no dogmatic assertion as to the superiority of one or another. Nearly every blue dye has at one time been recommended, and individual taste is more than likely to be at the basis of many proposals. We have tried a number of them, particularly alum hematoxylin, and the methylene blue complexes, but satisfactory results may be obtained with polychrome methylene blue, methyl blue and several green dyes. A thorough consideration of all the possibilities would be endless.

In general, it seems that the depth of the counterstain required varies according to the residual fuchsin in the tissues. We must confess a distaste for this residual dye and a necessity of employing a heavy counterstain to counteract it. In rodents particularly, the use of any of the methylene blue complexes dyes the granules

of the tissue mast cells intensely, which is a virtue in view of their often confusing acid-fastness. Very beautiful results are obtainable with these dyes.

Probably the simplest counterstain is methylene blue in a 0.1-1 per cent solution rendered very slightly alkaline with a few drops of ammonia. Only a few seconds are required and the depth is readily controlled by differentiation in a very weak acid alcohol. For color contrast with the red bacilli the color of the methylene blue dyes is probably superior to all others. For alcohol-fixed material it seems to give better results (or, rather, is easier to handle) than azure A, or azure II.

Hematoxylin (Erhlich's, Harris', or Delafield's alum hematoxylin) gives a sharper nuclear stain, more permanent than that of methylene blue. In exudative or necrotic lesions in which many cells are undergoing destruction it may be essential. Used alone in some tissues, particularly those with a surface epithelium, it may be adequate, but overstaining is needed to obtain a good contrasting color in structures other than nuclei. Bluing the hematoxylin is best done in running tap water for 20 minutes or more or in lithium carbonate. Hematoxylin is best used simply as a nuclear stain, with the use of another dye (methylene blue) as a general counterstain. We are not certain that it may affect the permanency of the acid-fast stain adversely.

It is possible, as has been recommended, to use the counterstain before the fuchsin. This is not difficult with hematoxylin, but it seems to us pointless.

FACTORS AFFECTING THE PERMANENCY OF ACID-FAST STAINS

When well stained the color of the bacilli in sections should darken slightly within a few days. There is no loss of definition and the color will probably be permanent. We have learned to mistrust the very brightly and brilliantly stained organisms, as they are more likely to fade, for reasons unknown.

If traces of alcohol remain in the mounted section the bacilli may be expected to fade. The ability of alcohol alone slowly to extract the dye explains also why sections must not be left standing in alcohol awaiting mounting, as well as the necessity of using several changes of xylol before mounting. No method is proof against these errors.

An acid balsam or dammar, or one from which the ethereal oils have not been removed (Unna) will bleach the bacilli as it dries.

The best stained and mounted sections will not resist undue amounts of heat which cause the dye to diffuse from the bacilli. Even the temperatures of the tropics may be sufficient to exert a deleterious effect.

OTHER STAINING REACTIONS OF ACID-FAST BACILLI

1. Methylene blue. He who copies Koch's original method of staining acid-fast bacilli with methylene blue will perhaps be surprised by its worth. The bacilli stain readily and well; the selection of a counterstain which affords good contrast and does not affect the color of the bacilli is difficult.

2. Methyl violet, crystal violet, Gram's stain, Much's stain, and so on. Methyl violet was used by Koch and Ehrlich to stain tubercle bacilli before Gram in 1884 devised his method, using the same dye. Acid-fast bacilli are stained by the methylated pararosanilines by either the acid-fast method or Gram's method. The tissues are more difficult to decolorize by the acid-fast method, but the bacilli are better and more permanently colored. Prolonged staining may be required, but acid-fast bacilli appear to be stained much more rapidly by these dyes than by the fuchsin. Lillie's variant of Hucker's formula is preferred to the aniline preparations:

Crystal violet	2 gm.
Alcohol, 95 per cent aqueous	20 cc.
Ammonium oxalate, 1 per cent aqueous to make ..	100 cc.

We have been unable to distinguish any differences in the color or staining power obtained with crystal violet and the redder methyl violets, which latter probably contain much crystal violet. Hematoxylin, combined with van Gieson's acid fuchsin-picric acid is on the whole preferred to safranin as a counterstain. It is almost certainly true that the wide popular use of fuchsin rather than crystal violet for staining acid-fast bacilli has been due to the want of a wholly adequate contrasting counterstain for the crystal violet, for the latter dye is superior to basic fuchsin in its

staining abilities, although not so much so to new fuchsin in comparable strengths.

3. Thionin. This dye stains acid-fast bacilli, metachromatically, a reddish color. It appears not to be of practical use.

4. Safranine, eosin, neutral red. Safranine has been recommended for staining lepra bacilli in sections. Eosin and neutral red will also stain the bacilli, but the colors of all these dyes are so much less intense and more transparent than that of fuchsin that their use seems uncalled for.

5. Janus green. This dye is probably the equal of fuchsin and crystal violet in its power of staining acid-fast bacilli. The difficulty again is the counterstain.

6. Formaldehyde produces a curious effect on bacilli stained with fuchsin, rendering them violet and highly resistant to decolorization, but not affecting other acid-fast structures, mast cell granules and the shafts of hairs, in the same manner. We have used the following procedure with success, when bacilli were most difficult to stain:

- (1) Carbol-new fuchsin 30-60 minutes, or longer.
- (2) Formaldehyde, 40 per cent, 5 minutes.
- (3) Acid alcohol until nearly decolorized, 15-20 minutes.
- (4) Formaldehyde, 40 per cent, a few seconds.
- (5) Counterstain in hematoxylin and van Gieson's stain.

7. When sections are treated with potassium permanganate some of the manganese oxides are deposited in the tissues, although the exact substances are not known. These are also deposited in acid-fast bacilli and are less readily removed from the bacilli by oxalic acid than from the tissues. We have seen bacilli fairly well demonstrated thus, although the effect on the tissues is harmful. In a less heavily impregnated state, however, some bacilli stain rapidly and intensely with fuchsin:

- (1) Potassium permanganate, 1 per cent aqueous solution, 5-15 minutes.
- (2) Carbol-new fuchsin, 5-30 minutes.
- (3) Acid alcohol, which will not wholly decolorize the section, 1 minute.
- (4) Potassium permanganate, 1 per cent aqueous solution, until the section is a definite brown.

- (5) Oxalic acid, 2 per cent aqueous solution.
- (6) Counterstain in methylene blue.

The bacillus of rat leprosy appears to be especially readily stained by this procedure, strikingly more so than the tubercle bacillus, human lepra bacillus and some other acid-fast organisms.

NON-ACID-FAST FORMS OF ACID-FAST BACILLI OCCURRING IN TISSUES

We have tried to emphasize that wholly erroneous conclusions may be drawn with regard to the occurrence of acid-fast bacilli in tissues, unless alcoholic fixation and adequate staining are employed.

When bacilli begin to undergo disintegration in the tissues, which usually takes place intracellularly, they stain less readily and may appear fragmented. In some lesions the cytoplasm of the cell may acquire an acid-fast cast, and in some others we have seen cells containing acid-fast granules or droplets, obviously derived from bacilli. But it seems to us that when acid-fast bacilli are destroyed it is never by the process of losing first their acid-fastness. It may be weakened, but ideal staining methods will still bring it out. This is another way of stating that if the Gram stain shows more bacilli than the acid-fast stain, then the fault is with the latter. All acid-fast bacilli are Gram-positive. When they are destroyed in tissues they lose both properties simultaneously.

RÉSUMÉ

The optimal staining of acid-fast bacilli in tissues involves fixation in an alcoholic medium, removal of mercuric deposits with iodine followed by alcohol and sodium thiosulphate, and staining in a 1 per cent solution of new fuchsin in 5 per cent phenol and 10 per cent methyl alcohol.

If a potassium dichromate fixative is used the period of fixation must be brief and the tissue thoroughly washed, and the section must be treated with potassium permanganate and oxalic acid.

Time and materials are wasted in trying to stain acid-fast bacilli in tissues subjected to prolonged passage in formaldehyde or decalcifying fluids.

To ensure the maximal staining possible under the conditions of fixation, the sections should be stained:

After alcohol fixation, at 20° C. — 2–8 hours,
 at 37° C. — 1–4 hours,
 at 60° C. — 30 min. to 2 hours, or
 at 90° C. (steaming) — 5 minutes;

After all other fixation, at 20° C. — 16–24 hours,
 at 37° C. — 12–16 hours,
 at 60° C. — 8–12 hours, or
 at 90° C. — 5 minutes.

Under good conditions bacilli may be adequately stained in much shorter periods of time than these. Bacilli not stained by fuchsin in the maximum times given will not be stained by further treatment. This is the point to which the duration of staining is best carried. Prolonged staining at room temperature is preferred.

New fuchsin as a dye for acid-fast bacilli is much superior to basic fuchsin.

When, in Zenker fixed tissues, fuchsin fails to stain bacilli resistantly, they may sometimes still be stained by Gram's stain, by the acid-fast method using crystal violet, or by the fuchsin-formaldehyde method here recorded.

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EXSTROPHY OF THE BLADDER WITH IMPERFORATE ANUS,
ABSENCE OF THE GREATER PART OF THE SMALL AND LARGE
INTESTINES, CONTINUITY OF THE DUODENUM WITH THE
COLON, ABSENCE OF THE LEFT TESTIS, EPIDIDYMIS
AND CORD, AND ENORMOUS HYDROURETER *

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On May 17th, 1936, a 17 year old colored multipara was admitted to the Maternity Pavilion, Hospital of the University of Pennsylvania, in active labor 2 months before term. Presentation was by the breech, and the birth of a malformed infant was followed by its death in about 10 minutes.

The body was that of a slightly premature male infant 39 cm. in length and 2000 gm. in weight. The head, thorax, back and limbs seemed to be normal, but most of the anterior abdominal wall consisted only of a translucent tough membrane (peritoneum? membrana reuniens?) that extended from the xiphoid and rib margins downward to the pelvis and was pressed forward, like a balloon, about 6 cm. in diameter, by the viscera behind it. There was no umbilicus; the cord, of which a generous portion was still present, divided several centimeters before reaching the body, each half being attached to the outer side of the membranous belly covering, and continuing over it until the skin margin was reached. The branch on the right side contained the umbilical vein and found its way to the liver; that on the left side contained a single artery.

There was complete exstrophy of the bladder, whose posterior wall was so completely everted as to form a dark colored rugose body that not only projected forward but, probably because of the pressure behind, downward as well, until its appearance and position closely resembled an enlarged scrotum. At the anterior, or superior, border of this everted bladder there was a somewhat ragged transverse slit from which a small quantity of greenish paste (meconium?) escaped. At the posterior, or inferior border, there was another less definite slit from which nothing escaped.

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The penis consisted of two short dark colored cylinders, placed side by side, thought to correspond with the corpora cavernosa, and a third, less definite cylinder below and to the right side, terminating in a knob — probably the corpus spongiosum with the glans. Below these structures were two folds of skin projecting laterally to the right and left, with a ridge between, supposed to be the halves of the scrotum and the intervening raphe. No testes were enclosed beneath these folds.

Behind these structures the perineum was entire, smooth, and without the usual dimple to mark the position at which the anus should have been formed.

There were no ureteral orifices on the lower lateral surfaces of the inverted bladder wall. The mucous membrane of the bladder did not meet with skin, but on each side there was an interval of about 4 cm. where the membranous inferior abdominal wall thickened greatly and became tough and elastic. Equidistant from the bladder proper, on each side, this thickened area presented a dark colored eminence of somewhat spongy appearance, as though the summit had been adherent to something that had been torn away. The probable nature of these eminences will later be brought out.

On each side of the rudimentary genitalia there was a hard, slightly elastic, somewhat pointed projection supposed to be the imperfectly formed and widely separated pubic bones.

The malformations thus far described were visible externally and consisted of: (1) a large congenital umbilical hernia; (2) exstrophy of the bladder; and (3) imperforate anus.

As soon as the abdominal cavity was opened a large cystic body at first thought to be a tumor escaped through the incision. It was about 8 cm. in diameter, thin walled and apparently multiloculated; it filled the left side of the abdomen from diaphragm to pelvis. Both kidneys were found in their respective positions and were of normal size and general appearance. The pelvis of the left kidney appeared normal but the ureter, just below the infundibulum, was considerably dilated. With the intention of introducing a probe into the ureter, a snip was made with scissors, when clear fluid escaped under pressure and the largest loculus of the tumor slowly collapsed. It was thus made probable that the supposed cyst was a dilated ureter and that the escaping fluid was urine.

Selecting a cyst at the opposite pole of the tumor, a snip was

made in it and the fluids escaping from both cavities were caught in separate test tubes. Subsequent examination showed both fluids to be urine. As the urine escaped the whole tumor mass gradually collapsed, showing that all of its chambers communicated. By gently drawing upon different parts of the cyst walls and carefully breaking up the external connections that bound the cysts together, it was finally determined that instead of a cyst or cystic tumor, the structure under examination was an enormously dilated convoluted ureter whose approximated twists had been united by external adhesions.

By what mechanism this dilatation had been brought about could not be determined, as a probe passed upward through the first snip made in the ureter entered the pelvis of the kidney without meeting with any obstacle. The pelvis and its infundibulum were normal; it was only the lower part of the ureter that had become enormously dilated.

When all of the adhesions had been separated so that the dilated tortuous structure could be sufficiently straightened, the probe easily found its way from the pelvis of the kidney to the lower termination of the ureter, which was not, however, in an ostium on the bladder wall, as is usually the case in exstrophy of the bladder, but in the spongy tissue immediately subjacent to the eminence of dark colored, thickened surface tissue described above.

The right kidney, its pelvis and its ureter were normal in size and appearance, but a probe introduced into this ureter found its way to the corresponding eminence on the right side and found no point of exit. As, however, this ureter was not dilated, the urine must have escaped through minute openings in the spongy tissue. Both ureters terminated in the same indefinite porous tissue in the respective lateral eminences on the external surface. It seems, therefore, not improbable that the superficial area of thickened membrane with the small polypoid inverted bladder in the middle, and the projecting boss on each side, represented the structures that should have formed the trigone of the bladder, and were largely made up of substance derived from the lower parts of the wolffian ducts.

Deep down behind the other viscera in the right flank, a testis, epididymis and spermatic cord were found. There was no corresponding structure on the left side.

The next striking abnormalities had to do with the alimentary canal.

The stomach appeared to be normally placed in the vertical position and was of about the normal size and shape. Beyond the pylorus was a short tortuous duodenum that continued into a thick walled tube 5 to 6 cm. in length, presenting an appearance closely resembling large intestine. It was almost white in color, and though not more than 5 mm. in diameter, was marked by grooves suggesting the valvulae conniventes. On microscopic section this structure proved to be colon. When a probe was passed from the stomach through the duodenum and on down through this short colon, it escaped through the slit-like opening mentioned above as occurring above the exstrophic bladder and between it and the imperfect abdominal wall — that from which meconium escaped.

The general direction of this short bit of gut was up and down, and beside it, almost in the midline of the abdomen, there was an ambiguous straight structure of cylindrical form whose direction was also directly up and down. At its free upper extremity, which was cecal and rounded, there was a kink, by which the first centimeter of its length was made to assume an obtuse angle with the remainder. The cylinder, almost white in color, was attached by a short straight mesentery. Microscopic sections made through this structure showed it to be perfectly formed small intestine, the mucosa having well formed villi. A probe introduced into its lumen passed upward to the cecal end; passed downward it escaped through the externally visible slit between the exstrophic bladder and the malformed genitalia. There were no other intestines, small or large.

The remaining abdominal viscera seemed to be without interest. The liver, gall-bladder, spleen, adrenals and pancreas were normal in size, shape and position.

The heart and lungs were of normal size, shape and position. There was a large thymus.

To the external malformations previously mentioned must now be added the following internal ones: (4) congenital absence of the greater part of the intestinal canal; (5) communication between the duodenum and the colon; (6) an isolated short length of small intestine opening externally; (7) enormous hydroureter; and (8) absence of left reproductive organs.

COMMENT

It is difficult to understand by what circumstances these malformations were brought about. It was remarkable that the lower three-fourths of a ureter could be so enormously distended without disturbance of the pelvis of the corresponding kidney and the occurrence of hydronephrosis. The absence of the reproductive organs of the left side might conceivably be the result of pressure atrophy caused by the heavy hydroureter mentioned above. It is difficult to imagine, but it may be possible that originally there had been a complete alimentary canal so disturbed by the pressure of the hydroureter that almost all of it had disappeared, and of the two vestiges of the intestine that remained, an abnormal ulceration of the duodenum into the colon resulted in one piece consisting of stomach, duodenum and ascending colon, and the other in an isolated piece of small intestine. But how did the vestige of the colon come to open externally above the exstrophic bladder and the vestige of the ileum to open externally below it?

The theories explaining the occurrence of exstrophy of the bladder do not seem adequate to explain how the trigone became spread out to the great extent of tissue seen in this case or how it came about that the ureters did not open on the sides of the bladder as usual but terminated in the peculiar spongy tissue described.

DESCRIPTION OF PLATE

PLATE 129

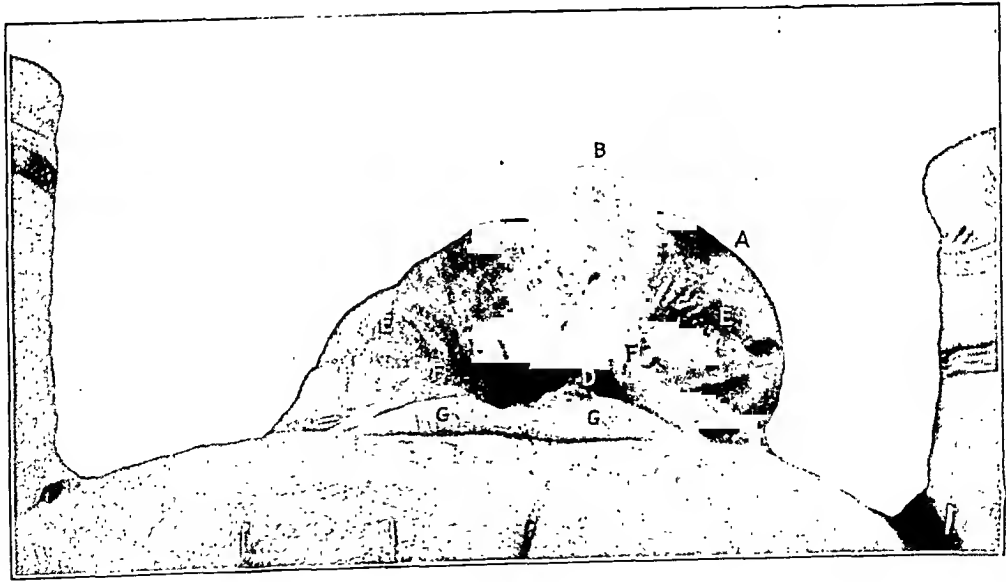
- FIG. 1. Gross photograph showing the translucent membranous enclosure of the abdomen, through which the viscera are visible near the center and in the midline below the dark posterior vesical wall rounded and digitate and suggestive of the penis or scrotum.
- FIG. 2. Rear view showing the prolapsed membranous belly wall at A, the everted posterior vesical wall at B, and the deformed penis at C. Above the dark penis is a fold of skin covering from side to side just below the coccygeal region, which is the imperfect and empty scrotum, D.
- FIG. 3. The exstrophic vesical region viewed from behind and below. A = the membranous abdominal wall; B = the projecting rugose posterior vesical wall; C = the glans of the malformed penis; D = position of the slit-like orifice through which a probe passed into the short piece of small intestine; E = spongy tissue at which the ureters terminated blindly; F = pubic bone widely separated; and G = scrotum.



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MALIGNANT GIANT CELL TUMOR OF BONE *

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INTRODUCTION

The question of the transformation of benign giant cell tumor of bone into sarcoma has been discussed for over 100 years. Lack of adequate pathological interpretation and especially adequate microscopy prevent modern evaluation of the statements of Cooper and Travers,¹ Lebert,² and Paget.³ Much prominence has been given to Nélaton's monograph,⁴ yet a careful perusal by Coley seems to show that Nélaton had but 6 cases of giant cell tumor which he personally treated, and that of 46 cases reported, mostly from the medical literature, but 14 were tumors of long bones. The follow-up of these cases was unsatisfactory; 4 individuals died postoperatively in the pre-Lister period, only 2 were traced for a 2 year period, 4 for a 1 year period, and the others were lost. Paget in 1853 called attention to the benign quality of giant cell tumor but left open an avenue of escape, apparently being by no means certain that some might not run a malignant course. In his *Surgical Pathology*, third edition, 17 years later, we find the same avenue open. One gains the impression that Paget may not have accepted the sarcomatous transformation of giant cell tumor but that he regarded the malignant tumors as something else. He states "nor have they, in general, any features of malignant disease, although myeloid structures have occasionally been found mingled with the ordinary structures of medullary cancer." He

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quotes instances in proof of the benign nature of myeloid tumors but draws his material entirely from tumors of the jaw. Pathological confusion appears obvious in one of Paget's cases — that of a farmer's boy with an enormous tumor of the calvarium, "believed to have originated in the effects of repeated blows on the head." From gross specimen, drawings of certain microscopic appearances, and from the clinical course, this tumor was clearly a meningioma which provoked much osteoplastic change.

Lebert first expressed himself of the view that all giant cell tumors were benign, but later altered his opinion. Virchow fully recognized the benign course of giant cell tumors of the jaw but agreed that giant cell tumors of long bones might yield highly malignant metastases. He calls attention to the fact that a tendency existed to regard such cases as complicated when viewed in retrospect, but incorrectly so. Virchow⁵ himself discarded the case of Gerlach⁶ in so far as to refuse to accept it as a tumor of medullary origin, but states that the case is nevertheless of value for the question of malignancy of myeloid tumors. Why he reaches that conclusion is not told us.

Virchow seems skeptical about Hutchinson's case.⁷ In this patient the tumor is said to have consisted of a mixture of "myeloid" and "fibro-plastic" elements. The primary tumor was located in the upper humerus. Its definite onset followed 14 months after fracture dislocation but no surgical interference was permitted until nearly 5 years had elapsed. Resection and axillary dissection were followed by rather prompt recurrence, fungation, cervical extension — said to be in "glands" — and death. Metastases were found in the lungs. In the infra-axillary and supra-clavicular "glands" (the present authors use glands in quotation marks because of a suspicion that the lesions were venous emboli), no giant cells were found and only "fibro-plastic" elements. The lung nodules contained giant cells with one to three nuclei. From the description these are probably malignant giant cells. The present authors see no reason from the available data to assume that this was other than a malignant change in a giant cell tumor. Virchow also doubts the case of Forster⁸ which was indeed re-surveyed with a follow-up by Wilks,⁹ and classed as "osteoid cancer combined with myeloid disease."

Virchow has less doubt in accepting the case of Henry,¹⁰ yet on

review of Henry's account the author himself seems to express misgivings for the first time as to the significance for prognosis of the myeloid cells. He writes that "the question now arises, whether they indicate anything more than that ossific changes are occurring in a tumor" and that "subsequent experience may enable us to determine the exact import of myeloid cells, but at present, it seems to me premature, to evaluate a characteristic which may after all only be accidental, into a test of a radical difference in the nature of a tumor." Finally, Virchow accepts without doubts the case of Wilks.¹¹ This seems strange for in this paper there is no evidence of microscopic examination of either primary, recurrent or metastatic masses. In fact Wilks stated that the naked eye characteristics of true myeloids were abundantly sufficient to distinguish them.

On reconsideration it would appear that the evidence on which Virchow based his acceptance of the occasional malignant course of giant cell tumors of the long bones is insufficient for present day standards of analysis although we do not doubt the truth of his conclusion.

Geschickter and Copeland¹² describe a case (JCB. N. 13714 — Dr. Dingman) where Bloodgood in 1924 had made a diagnosis of cellular but typical giant cell tumor on the basis of two curetted specimens, and of sarcoma with altered giant cells on the basis of an amputation specimen shortly thereafter. They regard the case as one of chondroblastic sarcoma of the femoral condyle rather than one of giant cell tumor. The patient rapidly developed pulmonary metastases and died within 6 months of the first symptom.

These authors similarly interpret the report of Stone and Ewing.¹³ Here the diagnosis of chondrosarcoma is apparently reached from a perusal of microphotographs. We ourselves are in possession of the pathological material and cannot find evidence of a cartilaginous element. The bone shown in the published photograph is not tumor bone but bone in process of destruction.

Bone Registry case No. 68, patient Mrs. K., treated by Wilson and Simmons, is accepted by Geschickter and Copeland as benign giant cell tumor. They find on clinical analysis that the patient died of pneumonia and cardiac failure. However, Dr. Wilson, who actually cared for this patient, tells us he feels certain the patient had pulmonary metastases. Ultimate interpretation of the

case is dependent on facts no longer obtainable. Geschickter and Copeland interpret the changes in the recurrent tumor of this patient as a healing reaction. Others viewed them as evidences of malignant transformation. Although the process is not marked, we find in the initial tumor certain "stromal" areas of confluent, very cellular pseudosyncytial mesenchymatous appearing cells which remind us of features seen in other tumors that have run a malignant course, and though not denying that the tumor was a giant cell tumor, our initial prognosis would have been guarded. It is interesting to note that the periosteum was found broken through at first exploration, probably as the result of failure to regenerate at the patient's advanced age. Moreover, everything that might be supposed to excite into accelerated activity a borderline tumor seems to have been done — four curettages, packing with resultant fungation of uncontrolled growth, and intratumoral implantation of radium. Infection was inevitable.

The case described by Augé and Roux¹⁴ was probably not a benign giant cell tumor. The patient was a male, aged 22 years. His initial symptoms were pain in the lower femoral region, relieved by rest. The pain was greatly accentuated by a fall 11 days prior to hospitalization. On admission the knee showed a rounded swelling, edema, local warmth, prominent veins, and a small effusion into the joint. The radiographic reports cannot be interpreted in terms of modern roentgen diagnosis. Fever was present and the process was thought to be osteomyelitis. After 9 days bed rest a pathological fracture occurred and amputation was performed. Within 2 months signs of pulmonary metastases appeared with pleural pain, "grippe," fever, emaciation, and bloody sputum; a scar recurrence grew rapidly, soft part metastases appeared and oliguria was noted. Autopsy showed pulmonary, pleuropericardial and renal metastases. Although the figures seem to show a primary benign giant cell tumor, the statement is made that it contained islands of cartilage and the pleural metastatic nodule is reported to have contained cartilage. The case is doubtless to be interpreted as chondrosarcoma with many epulis type giant cells. In a discussion, Delbet reaches a similar conclusion. At the same time he lays down certain rules for the diagnosis of benign giant cell tumors which, if followed literally, would surely make typical benign giant cell tumor a rare entity.

Geschickter and Copeland are probably correct in their interpretation of the case of Turner and Waugh¹⁵ as an instance of femoral thrombosis by benign giant cell tumor and not one of malignant giant cell tumor in the usually accepted sense. This case is similar to one described by Coley¹⁶ (J.McC., his case No. 11). We do not now regard this as a malignant giant cell tumor but as an example of local thrombotic recurrence of benign giant cell tumor. It is interesting to note that a radiologist skilled in diagnosis of bone tumors regarded this case as malignant from the beginning. Goforth's¹⁷ case provides no data on which a diagnosis of primary benign giant cell tumor may be made. The case of Finch and Gleave¹⁸ leaves much to be desired. The onset occurred with pain in 1915. Trauma was added in 1916, pathological fracture in 1917, which healed after exploration, and at that time a diagnosis of osteitis deformans was made. This is difficult to explain if the condition was a giant cell tumor. Pain recurred in 1919 and a diagnosis of giant cell tumor was then made. In 1925 a second fracture was followed by amputation, stump recurrence within a few months, and pulmonary metastasis. From the structure of the lung metastases an unqualified diagnosis of osteogenic sarcoma appears necessary. What the initial lesion was no one can say.

In Dean Lewis' case, reported by Geschickter and Copeland, all data on the original lesion were lost. Lewis is said to have believed the initial lesion to be a giant cell tumor. The metastases are described as bone-forming. One was curious in that it contained well developed marrow. This may be significant because of the fact that the present authors have twice noted evidence of hematopoiesis in areas of supposedly malignant giant cell tumors. We do not recall seeing such foci in osteogenic sarcomas, but this of course is not evidence. In a case to be discussed later King reports cells of "bone marrow type."

MacGuire and McWhorter¹⁹ report 4 cases of giant cell tumor where the histology is atypical and where local recurrences or metastases or both occurred. The structure and behavior of their case No. 34 corresponds to what we regard as malignant "transformation" of giant cell tumor. We believe their case No. 35 is of the same nature but find difficulty in interpreting the microphotographs. In their other 2 cases we find the data, as presented, unsatisfactory for conclusions.

The case of Dyke²⁰ is somewhat similar to that of Augé and Roux in the distribution of metastases. The patella is a most unusual site for the common giant cell tumor, unless it be of the tendon origin type. The microphotographs are chosen from small fields and although they apparently illustrate benign giant cell tumor of the usual variety one would like to see more of it, or have a more elaborate description of the histology before drawing conclusions. Dyke's report is followed by one by Orr.²¹ The latter publication is necessarily sketchy because the case is one resurrected from a museum specimen of 1898.

King²² reports a very satisfactory case. The tumor was located in the lower end of the radius. It had been present for 4 to 5 years. The radiographs were typically those of giant cell tumor and roentgen therapy was given. On later examination there was evidence of partial sclerosis commonly seen in giant cell tumor but also of cortical erosion and soft part extension. The radius was resected and a graft inserted. Local recurrence led to absorption of the graft; epitrochlear extension was followed by amputation, clear-cut X-ray evidence of pulmonary metastases and death. No autopsy was performed.

Sections were at first interpreted as benign giant cell tumor. At a later review malignant appearing areas were found containing active spindle cells and giant cells of both tumor and foreign body type. The figures from the epitrochlear tumor show loss of all suggestion of giant cell tumor. King regards the case as an example of malignant giant cell tumor of bone and specifically states that the term refers only to a malignant form of benign giant cell tumor, and does not refer to obvious osteogenic sarcomas which contain giant cells. With King's interpretation and terminology we are in full accord. We believe that there are tumors which show malignant features from some relatively early period but which are to all intents and purposes identical in nature with the benign giant cell tumor and which are distinct from osteogenic sarcoma in the usually accepted sense. The existence of such tumors must necessarily throw more responsibility of proof on those who assert that surgical or radiological interference with a benign giant cell tumor, or even pathological fracture, are the cause of its assuming malignant characteristics. This matter will be discussed later in connection with individual case reports.

Some writers call attention to the fact that there exists no proved case of pulmonary metastases from benign giant cell tumor where the structure of the pulmonary metastases was that of giant cell tumor. The metastases are said to show always the structure of osteogenic sarcoma. The authors have not had opportunity to study pulmonary metastases from malignant giant cell tumors but would suspect, from the structure of the histologically malignant primary tumors, that the metastases would not resemble the usual osteogenic sarcoma. Although to date no acceptable case of pulmonary metastatic tumor has shown the structure of giant cell tumor, it should not be surprising if such were eventually reported. Giant cell tumors are known to invade veins, spread to adjacent bones, appear in adjacent or more distant soft part tissues, and may well go farther without assuming an appreciably altered structure. In this connection one might recall the curious metastases of chorioadenoma destruens, the growth of chondroma into veins, venous invasion by uterine myoma, and rare distant metastases. Through the courtesy of Dr. Paul Steiner one of the authors has had the opportunity to study a case with massive pulmonary metastases from a uterine myoma. In this case * neither the uterine tumor nor the pulmonary metastases could be regarded as malignant from their histology alone. Malignancy is an attribute. The expression may refer to behavior and may refer to possession by the tumor of certain characteristic histological features. These phenomena are not necessarily always parallel. To risk diverging one might cite the infantile nevus. Many infantile pigmented nevi cannot be distinguished from malignant melanomas, but clinically they are benign lesions.

Efforts have been made to minimize the occasional malignant character of giant cell tumor on the basis of (1) confusion in initial diagnosis, mainly between chondrosarcoma and giant cell tumor, and (2) the development of the concept that the malignant tumor which arose in an otherwise innocent giant cell tumor was a "secondary" osteogenic sarcoma. The first is valid. The second is a matter of philosophical discussion. Adopting the terminology of the Registry of Bone Sarcomas, a system of classification which has been useful, but which possesses some inconsistencies, one might safely state that the malignant giant cell variant was an

* To be published in full by Dr. Steiner.

osteogenic sarcoma —a sarcoma *arising* from bone. We cannot see, however, that it is any more “secondary” than are many other tumors where one never thinks of such designation. Every tumor is secondary to something. Actual histology would suggest that some malignant giant cell tumors had more the characteristics of granulation tissue sarcomas than anything else. This would fit well with various notions concerning the histogenesis of giant cell tumor, namely a peculiar reparative process following necrosis of cortical bone under specific conditions. Mallory’s opinion²³ as to the essential reparative nature of the process designated as giant cell tumor has, we believe, received confirmation with the elucidation of the brown tumors of hyperparathyroidism. The mechanism of the radiation response of giant cell tumor is perhaps significant in this regard, for it is strongly suggested that radiation acts in these tumors toward reducing blood supply and permitting recalcification, rather than destroying tumor cells in the manner frequent in malignant tumors. It is also interesting that the last comprehensive study of the origin of the epulis type of giant cell in bone, that of Zawisch-Ossenitz,²⁴ reemphasizes the origin from penetrating endothelial sprouts. She reports solid endothelial sheets splitting off from invading capillary endothelium. Of course the interpretation is not new.

In a more recent single case report Puhl²⁵ attacks the theories of the essential reparative origin of giant cell tumor. He offers as substitute the statement that these tumors are dysontogenic lesions —tumors of embryonal mesenchyme, the mesenchymal cells being capable of differentiating in multiple directions, with the formation of giant cells, osteoid and cartilaginous tissue. Puhl is undoubtedly describing a case of chondromatous giant cell tumor. Admitting the possible correctness of his interpretation of the origin of chondromatous giant cell tumors, we question the advisability of assigning all giant cell tumors to similar origin. In several cases of malignant giant cell tumor we have noted resemblance of the tumor cells to condensed atypical mesenchyme. One must, however, consider the possibility of dedifferentiation and reversion in the production of such pictures as well as dysontogenesis. The exact mode of origin of the average giant cell tumor will probably await solution until opportunity arises, probably by accident, to see the lesion in its very early stages. It is difficult to

conceive of dysontogenic origin of the Brauntumoren of hyperparathyroidism.

Puhr ²⁶ emphasizes a reticuloendothelial origin for benign giant cell tumors. We cannot see that the evidence is especially clarifying and quote the paper only because the theory is of interest in view of the structure of certain of the malignant tumors in our own series.

In selecting the following cases for detailed report, efforts have been made to exclude cases of doubtful significance. Only when material from the initial curettage and from subsequent specimens, in which no possible doubt can exist as to the malignant quality of the process, is available for review is the case regarded as suitable for presentation. If, however, the first material available for study comes from a *second* operative procedure, but still shows the lesion to be a giant cell tumor, subsequent course proving the lesion to be malignant and with histological proof of change of character, the case may still be included since it cannot be assumed that a recurrence of an initially malignant tumor will take the form of a benign giant cell tumor. Typical roentgen evidence of pulmonary metastases and the death of the patient are accepted as proof of malignant character and autopsy confirmation is not considered essential. Strict exclusion of certain material robs the series of several cases where no doubt exists in our own minds as to facts, but it is our feeling that the inclusion of such cases would add no information.

One case is included, although it is considered as malignant from the earliest available material. It is included because it is believed that, like King's case, it is a malignant giant cell tumor and not the usually accepted osteogenic sarcoma.

CASE REPORTS

CASE I. H.C., male, aged 27 years, applied to the Memorial Hospital on June 1, 1929. He complained of pain largely confined to the popliteal region on the left, beginning 9 months prior to admission. There was no history of trauma. The joint had been swollen and inflamed. Motion was painful. The swelling and pain subsided at intervals only to recur. He walked with little difficulty. When the pain was severe, flexion was incomplete. On admission to the clinic no swelling was evident. Deep pressure failed to elicit pain but motion was limited. The patient presented radiographs which showed a large, multilocular cystic growth of the lower femoral region regarded as characteristic of giant cell tumor. The patient received X-ray treatment but since the

size of the portal is not mentioned the dose cannot be calculated. It was apparently not excessive. After treatment the early films are said to have shown improvement. The patient failed to report to the follow-up clinic until about 7 months had elapsed, when he returned with a pathological fracture which had occurred in bed. He was placed in a Balkan frame. Additional X-ray therapy was given and by April 30, 1930, the patient was using a walking Thomas caliper. Local swelling and tenderness following fracture had regressed. One month later radiographs were reported as showing suggestion of further decalcification and some reactivity of tumor. Thereafter films made about every 2 months were reported as showing little change. In October, 1931, the patient had a curettage with implantation of fat pad at another hospital. No drainage was done. About 1 month later the patient became febrile, the tumor showed marked evidence of local recurrence, and amputation was performed in January of 1932. The tumor rapidly recurred in the stump, pulmonary metastases were demonstrable, and the patient died on April 1, 1932.

Comment: It is difficult to assign the blame for the behavior of this tumor. Some would incriminate curettage, others the X-ray followed by curettage; some would suspect the influence of the fat pad, others the pathological fracture. We suspect the tumor itself, for reasons which will appear later. However there was rapid alteration after curettage and one may be justified in assuming the attitude *post hoc ergo propter hoc*.

Material from the curetted specimen (Fig. 1) comes from five different areas. The structure is essentially the same throughout. The giant cells are very large, some containing as many as 100 nuclei of uniform size and structure. They do not appear related especially to areas of blood pigment, hemorrhage or blood lakes. There are faint traces of dead bone in process of decalcification. No cartilaginous or myxomatous tissue is seen. In some areas the "stromal" cells are a trifle atypical, being more spindle shaped and less polyhedral than usual. No sharp demarcation can be made out, however, between these spindle cells, the polyhedral cells, and the smaller giant cells of probably recent formation. On long search, although mitoses are numerous in spindle and polyhedral cells, no atypical mitoses can be found. In some areas the spindle cells occur in hyaline areas no different from those of reactive fibrosis. A few well formed vessels are seen but for the most part the vascular channels are lined by single rows of endothelium. In some no endothelial lining can be made out. Hemorrhage has occurred in the interstices of the tumor and old blood pigment is found. There are occasional xanthomatous foci. In some areas

giant cells become sparse and spindle cells more prominent. There is a strong suggestion of vasoformative properties in these spindle cells and they appear inseparable from the capillary endothelium. Where the spindle cells are numerous they seem to grow in pseudo-syncytial fashion but cell boundaries are nevertheless present.

In the specimen obtained at amputation (Figs. 2 and 3) no cells of the epulis type are seen. Many giant cells occur but their nuclei are few in number, are very large, pale and vesicular, and some contain from seven to eight nucleoli. Where nucleoli are fewer they attain enormous size; some are as large as an entire normal plasma cell. Many atypical mitoses are seen. There are numerous degenerative mitoses where the cytoplasm is filled with coarse irregular chromatin granules. Some cells contain a single, large hyperchromatic nucleus, itself as large as a small epulis giant cell. Between the giant cells are large fusiform or polyhedral cells with rather clear cytoplasm, large vacuolated nuclei and giant nucleoli. Many show mitoses. All transitions between these fusiform and polyhedral cells and the tumor giant cells are found and there is a suggestion that the giant cells arise both by accretion and through multiple mitosis. Where the cells are more sparsely distributed a distinct endothelial character is noted. The tumor looks angioblastic, although this property is less marked than in other instances to be illustrated later on.

We can see no reason to call this tumor secondary. To do so arbitrarily creates division where none can be shown to exist. We believe the term "secondary" is a loose one. It might suggest that the lesion was of a fundamentally different histogenic type. This we do not believe. It is a continuation of the same underlying process in aggressive neoplastic form. "Secondary" might mean that a second cause or stimulus was operative. This may be quite possible. In fact, in most instances of malignant transformation of giant cell, tumor is strongly suggested.

CASE 2. L.S., male, aged 35 years, applied to the Memorial Hospital on Aug. 24, 1937. About 1 month before admission he began to experience discomfort about the left knee joint. Shortly thereafter he noted pain and swelling, and some tenderness on pressure. He consulted a physician who treated him under a diagnosis of arthritis, with no relief. Radiographs were then made and the patient was referred to the bone service at the Memorial Hospital. The lower end of the femur was involved by a destructive growth which extended from the articular surface of the right condyle upward for a

distance of 7 to 8 cm. The region was trabeculated. The cortex was thin and not perforated. The outer limits of the tumor were sharply demarcated, especially upward. It had the appearance of a giant cell tumor.

The patient was treated by curettage, washing out the cavity with zinc chloride solution in the usual manner, followed by Dakin's, and the wound was closed without drainage. Convalescence was at first uneventful but the patient complained of much more pain than usual about the time of his discharge. There was spasm of the hamstring muscles. On Oct. 10, 1937, blood was aspirated from the joint cavity. Roentgenographs at that time showed evidence of active extension of tumor accompanied by considerable increase in bone destruction. The lung fields were clear. Amputation was advised and accepted. On Jan. 10, 1938 the patient complained of pain in the chest. From then on, the downhill course was rapid; there developed metastases in the tenth thoracic vertebra, transverse myelitis, pulmonary metastases, and death occurred on March 14th.

Comment: The initial diagnosis of giant cell tumor was made by aspiration and from the aspirated material alone a note was made that the "stroma" was unusually cellular. From the curettings (Fig. 4), the tumor was reported as giant cell tumor with the reservation that its benignancy could not be guaranteed. There were numerous typical epulis giant cells with the characteristic, small polyhedral cell stroma. Hemorrhage had occurred with deposition of blood pigment. The blood supply consisted of widely dilated capillaries mostly with an endothelial lining of a single row of cells. Some vessels seemed to lack a complete endothelium and the tumor was telangiectatic. Hemorrhage had occurred in the interstices of the tumor quite recently but this was doubtless the result of the curettage. Old strands of fibrous tissue crossed the mass. There were traces of bone in process of decalcification. No cartilage or myxomatous tissue was seen. In one area (Fig. 5) the giant cells were scanty, nuclei less numerous, the cells were small, and there was a marked proliferation of cells of the "stromal" type, rather larger, slightly more ovoid, and slightly more hyperchromatic than the usual stroma cells. There mitoses were numerous but not atypical. These cells lined in part vascular channels without definite endothelial walls, although an occasional flattened cell resembling endothelium could be seen. Demarcations between individual cells were often indefinite. No tumor giant cells could be identified. On the basis of this area we refused to state that the tumor would run the course of the usual giant cell tumor but likewise refrained from calling it other than giant cell tumor. In examining the suspicious area Dr. Ewing stated that he

had previously seen such areas in giant cell tumors that had not run a malignant course. Nevertheless the atypical area fully justified our suspicions.

It is important to note that suspicious features in this tumor were found within a short time after known onset, prior to any interference, deliberate or accidental. In the amputated specimen we found an extremely destructive, soft, pulpy hemorrhagic tumor involving the lower end of the femur, including the epiphysis and lower 5 cm. of shaft. There was a pathological fracture, said to have occurred during or shortly after operative handling rather than prior to amputation. The tumor had broken through the cortex and a bulky soft part mass was present. The total bulk of tumor was about 13 by 13 by 10 cm. The joint cavity was invaded and filled with blood. There was an upward extension of tumor between the deep muscle planes. The gross diagnosis was aneurysmal giant cell tumor. In sections, only one area examined shows the characteristics of benign giant cell tumor. The others consist of tissue resembling the suspicious appearing area of the curettings. Cells are polyhedral or ovoid; nuclear-cytoplasmic ratio is disturbed, nuclei being unusually large. Growth appears to be largely syncytial and the tissue suggests a cellular, atypical condensed mesenchyme. The circulation is almost entirely telangiectatic. Giant cells of the epulis type are exceedingly rare. Mitoses are numerous; none appears atypical. The mode of growth varies from place to place. In some areas (Fig. 6) it is almost epithelial with cells appearing in sharply demarcated sheets. Such areas are also seen in endothelial tumors. Where hyaline fibrous tissue is being invaded it is impossible for us to separate satisfactorily the tumor cells from either fibroblasts or endothelium. Thus the tumor possesses characteristics seen in granulation tissue sarcomas. No tendency to form bone, cartilage or osteoid tissue is found. We class this case as one of primary, malignant giant cell tumor of bone.

CASE 3. G.G., male, aged 39 years, applied for treatment at Memorial Hospital on Feb. 15, 1929. He had had a curettage with packing of the cavity by gauze, at another hospital, of a typical giant cell tumor of the lower right femur, mainly the external condyle. Despite treatment, successive radiographs showed increasing destruction up to the time of the institution of X-ray treatment at the Memorial Hospital. From that time on, over a period of about 1 year, reports from radiographs indicated some increased bone

density, interpreted as a healing process. After about 1 year, however, films showed evidences of reactivated disease. Amputation was done on May 27, 1931. By September of 1932 a mass was palpable in the right groin. The mass extended into the abdomen. The patient complained of severe pain which finally necessitated chordotomy. On May 3, 1933, pulmonary metastases were found and on May 11th the patient expired.

Comment: Through the kindness of Dr. Jaffé we have reviewed the sections of the original tumor. Material from several different areas was interpreted by Jaffé and ourselves as typical benign giant cell tumor. There are no signs of bone production, atypical stroma, cartilage, or myxomatous areas. Sections from the amputated specimen 4 years later show a purely destructive, non-ossifying, highly malignant appearing sarcoma. The cells are loose, round or ovoid, and contain one or more large hyperchromatic nuclei and giant nucleoli. Reticular structure is absent. There is no tendency toward the formation of long spindle cells and no epulis giant cells are seen. There are numerous mitoses, some atypical and multiple. The circulation is telangiectatic. An aspiration biopsy from the inguinal mass also showed a malignant tumor.

This tumor may have been malignant from its onset but the burden of proof must rest on those who refuse to accept the contrary evidence.

CASE 4. D.K., female, aged 28 years, was first treated by Dr. Lewis Gregory Cole in 1931 for what was regarded as a typical benign giant cell tumor of the right lower radius. She received sufficient radiation to control the usual giant cell tumor, but about 9 months later there was roentgenographic and clinical evidence of recurrence. More radiation was given but the process remained uncontrolled. Hence a curettage was performed and the cavity was swabbed out with carbolic acid. Within 4 months the tumor had again recurred and a second curettage was done. Altogether the tumor was curetted on six occasions (in 1931, twice in 1932, three times in 1933). Additional radiation was given between the fifth and sixth curettage and after the last curettage.

Comment: Material from the earlier specimens shows a typical benign giant cell tumor, containing numerous giant cells of the epulis type, without undue vascularity, and with no unusual alterations in the character of the tissues between the giant cells. Material from the fourth curettage is extremely vascular and the giant cells are less numerous.

For the first time a distinct alteration was observed in material from the last curettage and then, especially since the tumor had

fungated, an amputation was performed. The giant cells in the amputated specimen are of two types but suggestions of transition stages are observed. Large, typical epulis type cells predominate but there are numerous smaller giant cells with few nuclei, sometimes only two or three. In the intermediate type of giant cell can be seen in addition to the usual nuclei of the benign giant cell type, one and sometimes two very large, hyperchromatic nuclei with correspondingly large nucleoli. Malignant giant cells are of the usual type, with few large nuclei and with giant, atypical mitotic figures. Giant mitoses are found in the intervening tissue. In some giant cells there is a suggestion of growth by fusion of nuclei, the resultant nuclei resembling those of megakaryocytes. We can not be certain that some such cells are not megakaryocytes, especially since in addition to all of the features found in the last curettings, accentuated only in degree, in the amputated specimen there is distinct evidence of blood formation (erythropoiesis).

Were this tumor anything but a giant cell tumor it is most remarkable that six curettages were necessary before its malignant characteristics were detected. The patient has nearly passed the 5 year period of freedom from disease.

CASE 5. M.L., male, aged 44 years, applied to the Memorial Hospital on Nov. 8, 1933. His history stated that he had had pain of sudden onset in 1926, located just below the right knee. At that time the lesion was considered a tibial bone cyst. Operation was advised but refused. The pain lasted about 3 weeks and then subsided. It did not recur until about a year before admission to the Memorial Hospital. In August 1933 the lesion was curetted at another hospital. The material was diagnosed giant cell tumor. Pain persisted and 3 months later a second curettage was performed. At both operations the wound was closed tight, without drainage. Although the early radiographs were considered those of a bone cyst or giant cell tumor, later films taken in January, 1934, were considered as showing that the process had become malignant. Amputation was then done. Only 3 weeks after amputation, disease was evident in the stump and a mass was palpable over Scarpa's triangle. In May of 1934 evidence of pulmonary metastases appeared in chest films. The recurrent lesions resisted roentgen therapy. The patient died in July of 1934.

Comment: Sections from five different blocks of the curettings obtained at the second operation showed benign giant cell tumor. Review of these sections shows benign giant cell tumor. Giant cells are of the epulis type. Nuclei are small, uniform, and very numerous. The stroma cells are perhaps a trifle more spindle shaped than in some instances of giant cell tumor. Some appear

to line blood spaces, interrupting the continuity of the endothelial layer. The lesion is very vascular. It is impossible to separate the capillary endothelium and perithelial cells from other elements, and blood vessels, as is usual, form an integral part of the process. There are old areas of hemorrhage and masses of blood pigment which probably date from the first curettage. There is no evidence of cartilage or myxomatous tissue. After long search a single giant cell was found with a nucleus and nucleolus much larger than normal. The cell appeared, however, degenerated.

During the 2 months that elapsed between the second curettage and the amputation the curetted cavity had partly filled with fibrin, but a mass of recurrent tumor 6 cm. in diameter had developed in its anterior half. This mass invaded the soft tissues; an upward extension penetrated beneath the patella and extended on both lateral aspects nearly to the popliteal space. In a small area this upward extension invaded the cortex of the femur. The curious outlines of the recurrent mass suggested invasion of veins. There is a marked structural change in the tumor, a change of considerable significance. One portion consists of dilated blood vessels containing leukocytes but essentially no red blood cells. These vessels are surrounded by perithelial spindle shaped cells which merge gradually into sclerosing fibrous tissue without definite demarcation between the perithelial cells and the fibroblasts. It has many characteristics which would lead one to regard it as neoplastic granulation tissue. These capillary channels merge imperceptibly with channels lined by large, thick, irregularly fusiform, very hyperchromatic, malignant tumor cells (Fig. 7). The nuclei are large and mitoses are numerous. About some of the vessels the perithelial cells are arranged in whorls of malignant appearing cells inseparable from similar cells lining the channels. Where neoplastic vessels are less numerous the interstitial tissue shows fibrosing tendencies and ranges in appearance from cellular fibroma to spindle cell sarcoma. Epulis giant cells are no longer found. Taken as a whole the structure is that of malignant granulation tissue sarcoma.

The long history in this case is very much against the idea that there was anything malignant about the initial lesion. We cannot avoid holding the suspicion that this malignant tumor arose in a benign lesion after multiple curettages.

CASE 6. Material from the case of J.N., reported in detail by Stone and Ewing¹³ is still available for study. We have reviewed this material in view of the assertion of Geschickter and Copeland that the tumor was not a giant cell tumor but a chondroblastic sarcoma. We are unable to find evidence that it was a cartilaginous tumor.

Comment: Since the case was reported in considerable length by Stone and Ewing we see no reason to duplicate the report. We find the early sections typical of giant cell tumor. The bone formation reported is a poorly developed calcification in an area of not very cellular hyaline osteoid tissue and is fully consistent with processes that may occur at the periphery of giant cell tumors or in pure inflammatory disease of bone. The "stroma" is not very cellular and the intercellular substance is quite fibrous, in some places almost keloidal in character. Although from the material remaining we are unable to trace the evolution of the malignant change in the recurrent tumor, material from the amputated specimen is still available. The malignant tumor present is similar to those described in other instances of this same change. The cells are loose, spindle or polyhedral elements, arranged in syncytial or pseudosyncytial fashion, rather delicate and hydropic appearing, and of an appearance suggesting that of condensing mesenchyme. Giant cells of the epulis type are absent. The tumor is quite different from known varieties of true osteogenic sarcoma.

CASE 7. E.M., female, aged 18 years, entered the Memorial Hospital on March 20th, 1928. Seven months prior to admission she first noted painful swelling of the right knee. This became progressively worse and she consulted a physician who performed a curettage after the roentgen diagnosis of giant cell tumor of the tibia. This curettage was not complete because of failure to secure hemostasis. Three weeks later a second curettage was likewise unsuccessful for the same reason. The patient was then referred to the Memorial Hospital. On admission the wound was found filled with gauze packing and was obviously infected. Radiographs taken after admission were indefinite; the tumor was considered malignant but it was also stated that an infected giant cell tumor, recurrent after curettage, could present the same features. Under external radiation, for a short period, the tumor fungated. Attempts to control growth by caustics failed, infection increased, and on May 26th, 1928, the leg was amputated despite roentgenographic signs of pulmonary metastases. The patient died 3 months later with extensive pulmonary disease.

Comment: The first sections show in our opinion a giant cell tumor, but like King's case and others of our own, we believe there were already definite evidences of malignant tendencies. In

fact there are areas in the first specimen that appear just as malignant as does the material from the amputation. Although the diagnosis of giant cell tumor is accepted we cannot say that it was ever benign. At the same time, in none of its characteristics does it resemble the usual osteogenic sarcoma. This case is carried in the registry of bone sarcomas as an osteogenic sarcoma but we feel that such tumors belong in a category by themselves.

Sections from the amputated leg show a tumor of pleomorphic structure (Fig. 8). There are large numbers of giant cells of the type seen in benign giant cell tumor. Some of these lie free in spaces. Some line vascular channels. Some lie free in vascular spaces. Many of the giant cells are continuous with reticular, loose, delicate appearing edematous tissue resembling mesenchyme. The reticular tissue passes, without lines of demarcation, over into small fusiform cells resembling fibroblasts. Some of the reticular cells are continuous with structures that resemble primitive vascular channels; the latter are lined by reticular tumor cells, interspersed with typical multinucleated giant cells. Maturation of fibroblasts and production of collagen are present to a very scanty extent. At intervals, among the loose reticular cells, a markedly hyperchromatic cell with a deeply staining ovoid nucleus is seen. Such cells are also found at intervals mingled with the reticular cells lining the vascular channels. Some contain central vacuolar spaces resembling those of primitive vascular channels. There is evidence that these hyperchromatic cells multiply by atypical multiple mitosis, producing large giant cells with several hyperchromatic, large irregular or giant lobular nuclei. Occasional cells of this type are seen in the vascular lumens. The tumor on the one hand definitely forms blood channels and, on the other, fibroblastic elements that are associated with the laying down of fine collagen fibers. Thus it resembles an angioblastic granulation tissue sarcoma of a peculiar type. In no areas are ossifying tendencies observed.

DISCUSSION AND CONCLUSIONS

We have been unable to arrive at a satisfactory descriptive term for these malignant giant cell tumors. It is perhaps best to retain the designation "malignant giant cell tumor" since it carries at least a definite connotation. Efforts to establish hard and fast lines

of distinction in cells involved in bone development have made the description of the histogenesis of bone most complex, and through the ultracytological analyses of various histologists cells have acquired individualities which they probably do not merit, or merit only in a transient sense.

We are unable to separate the cell elements of giant cell tumors from the connective tissue cells and vessels which are involved in the histogenesis of bone and which evolve in different directions dependent on the physicochemical conditions of the period. We feel much sympathy with the views of Moschcowitz,²⁷ as expressed in his paper on the relation of angiogenesis to ossification, and see many similarities in the development of malignant giant cell tumors. We venture to doubt that one can specifically state that a giant cell tumor is a tumor of giant cells, intervening connective tissue cells, or angioblastic elements, or that the malignant giant cell tumor is a sarcoma of giant cells, angioblastic elements, or an endothelioma or a granulation tissue sarcoma, since we find great difficulty in separating the elements of the tumor into permanent entities. In our own cases no true bone formation has been observed and yet it would surprise no one if a tumor with this evolutionary pattern should appear. Despite the tendency, which we also have followed, to reject as giant cell tumors of malignant type those tumors where cartilage has appeared in the metastases, still their rejection may not be necessarily warranted.

Thus the form assumed by the process known as giant cell tumor will be found to depend on the nature of the circumstances, physical and chemical, which have initiated the process, plus the extrinsic factors that interfere with its normal evolution. Until better understood, the interpretation of giant cell tumor and its malignant evolution must remain in a speculative phase.

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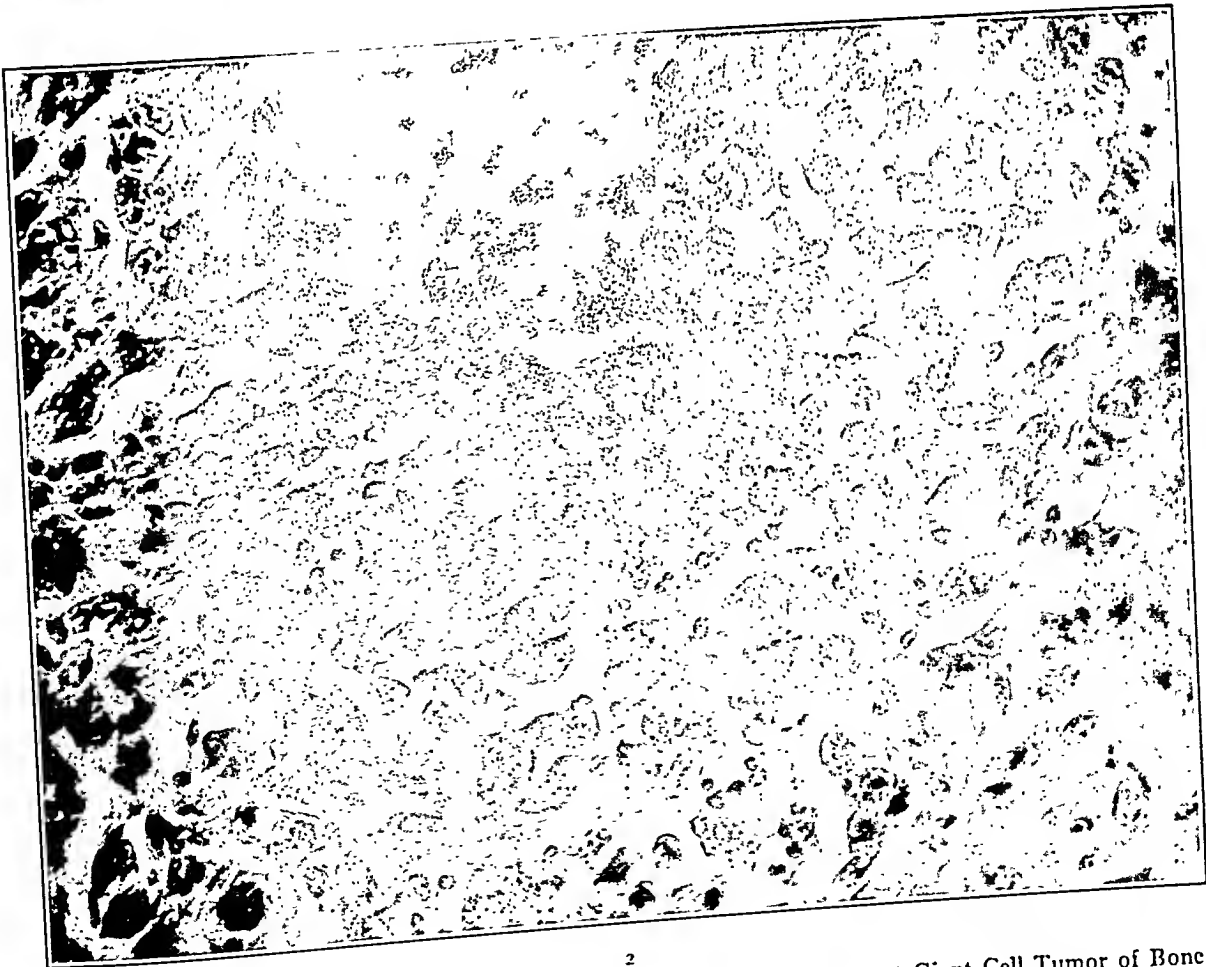
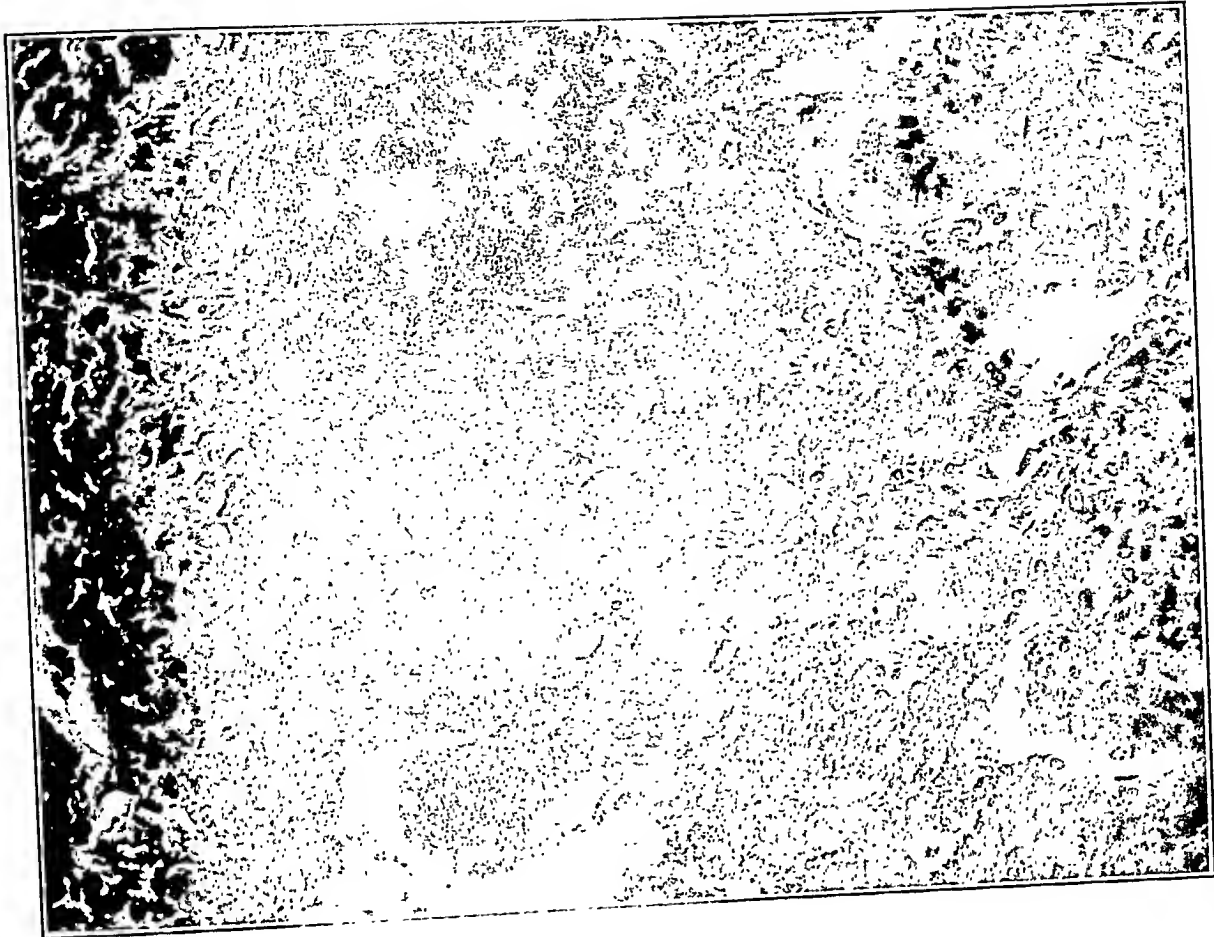
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DESCRIPTION OF PLATES

PLATE 130

FIG. 1. Typical benign giant cell tumor.

FIG. 2. Malignant recurrence of the tumor illustrated in Figure 1.

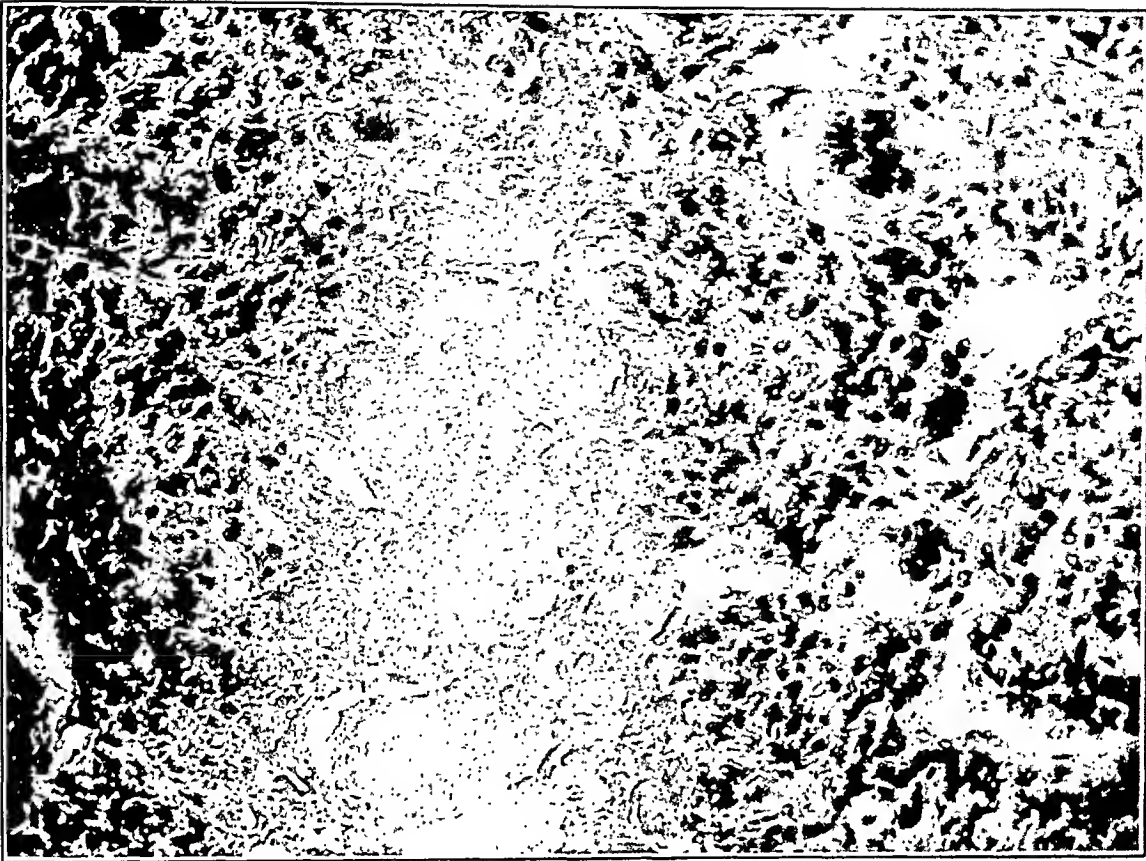


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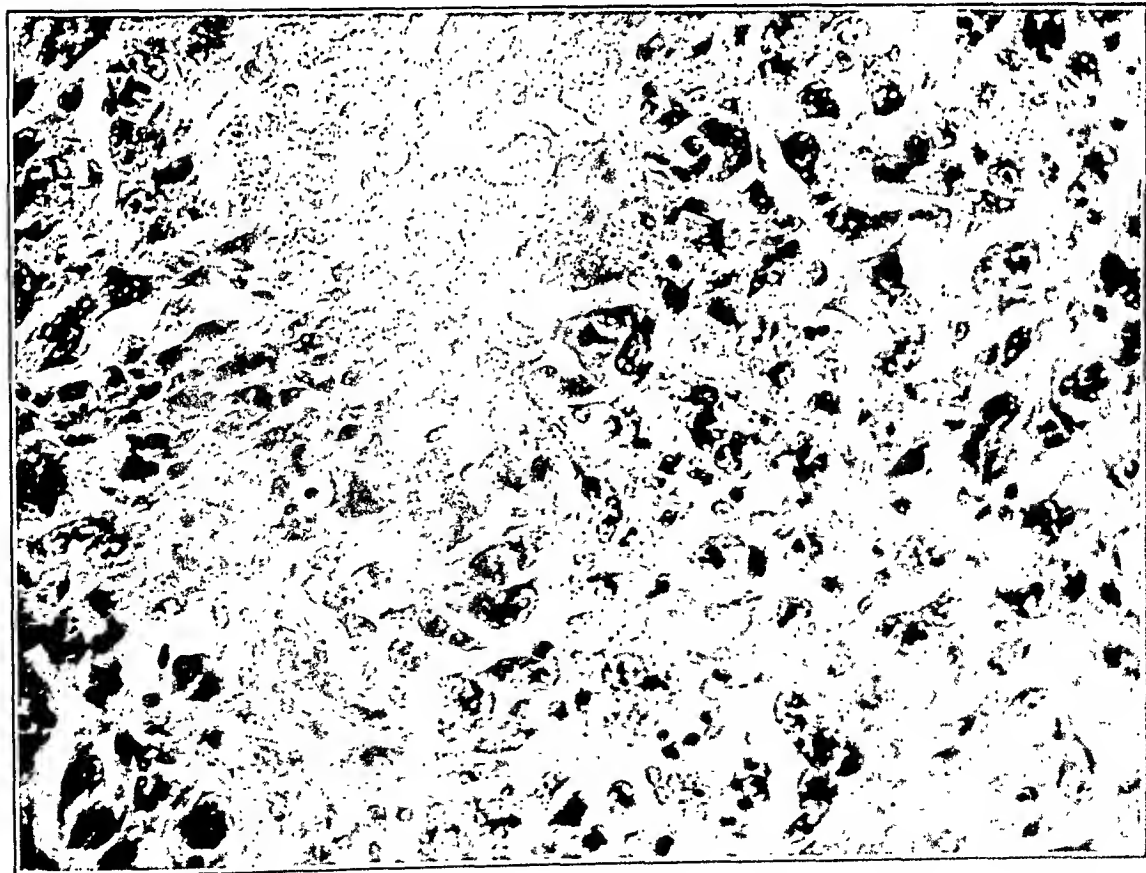
PLATE 130

FIG. 1. Typical benign giant cell tumor.

FIG. 2. Malignant recurrence of the tumor illustrated in Figure 1.



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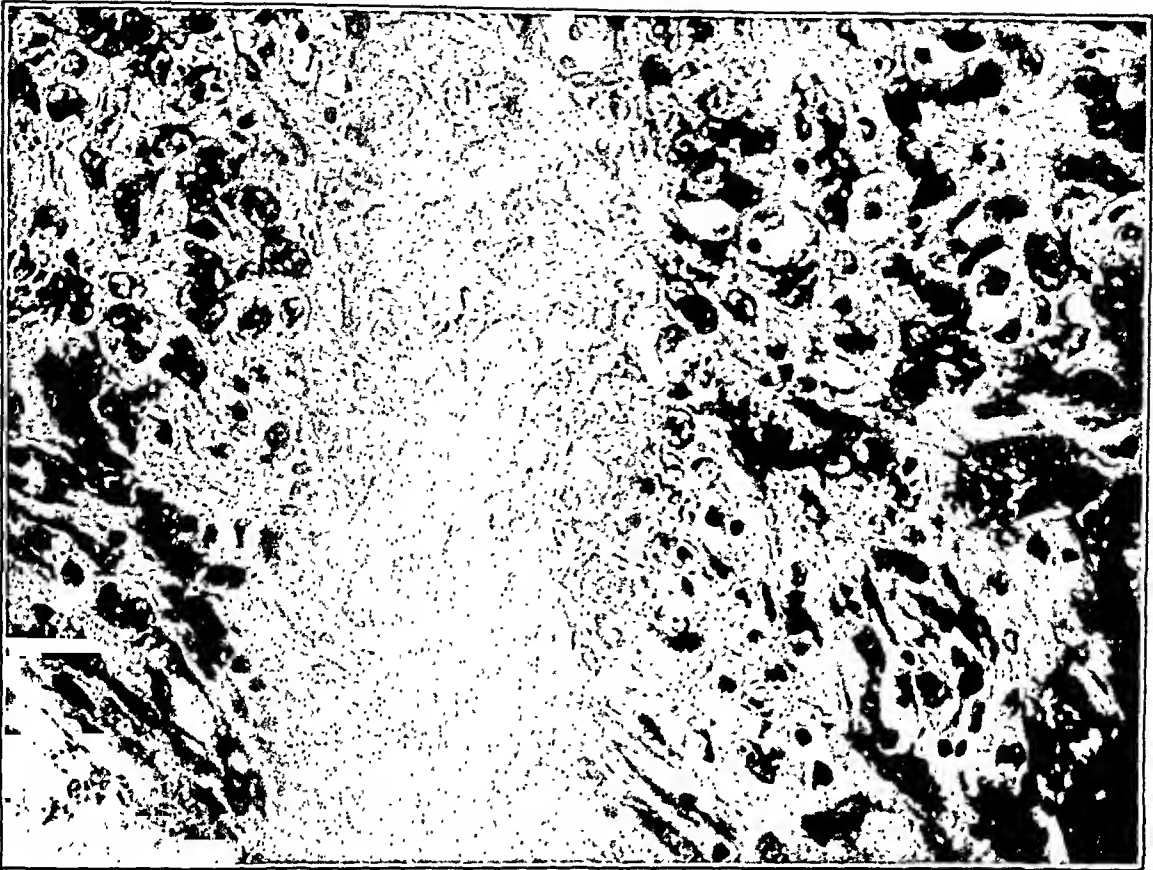


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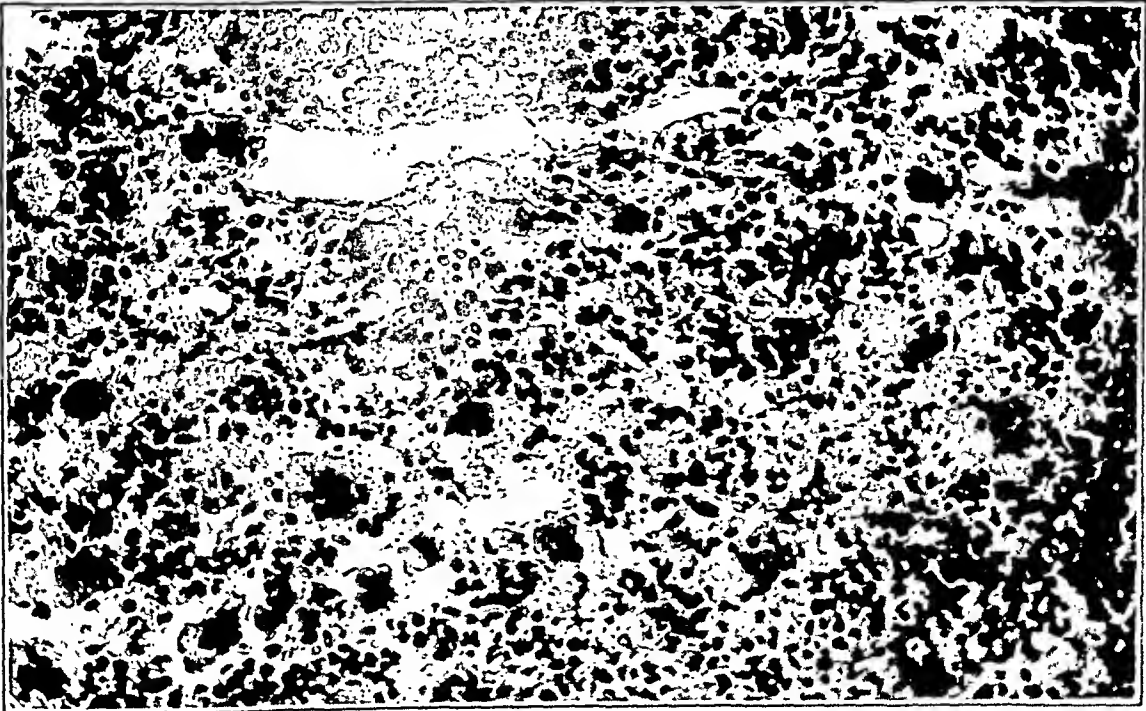
PLATE 131

FIG. 3. Malignant recurrence of the tumor illustrated in Figure 1. Syncytial cells growing in a manner suggesting the growth of endothelium.

FIG. 4. Typical benign giant cell tumor.



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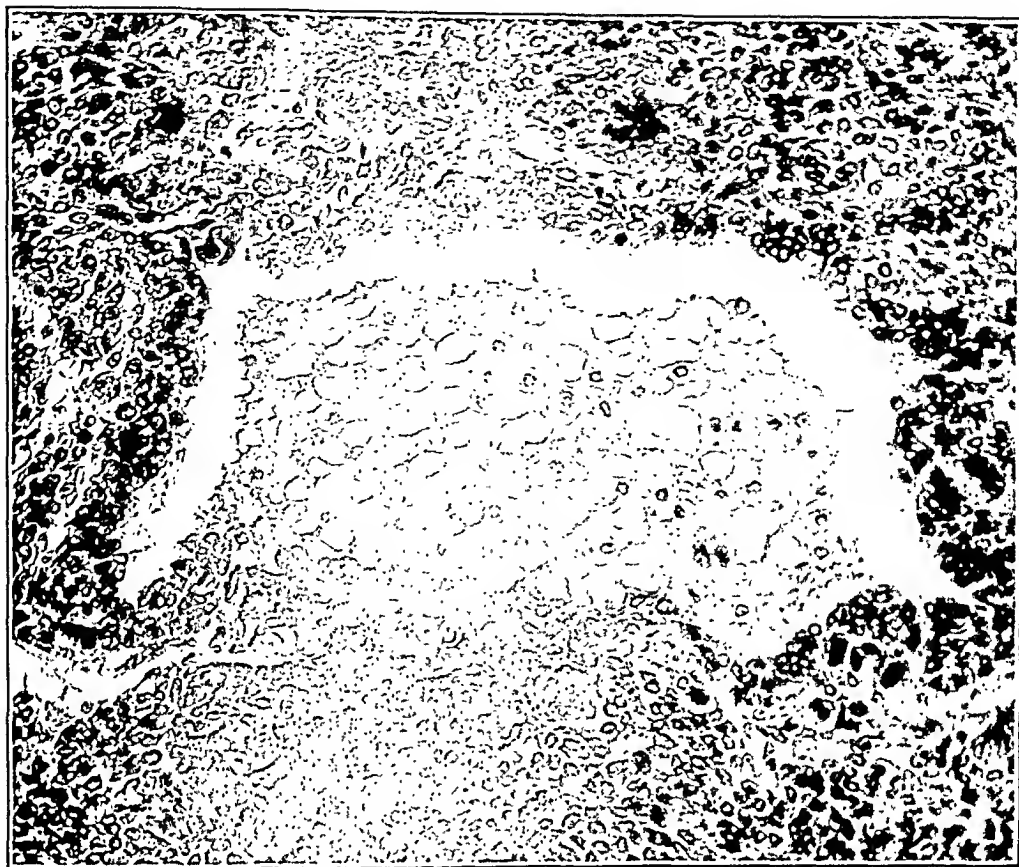


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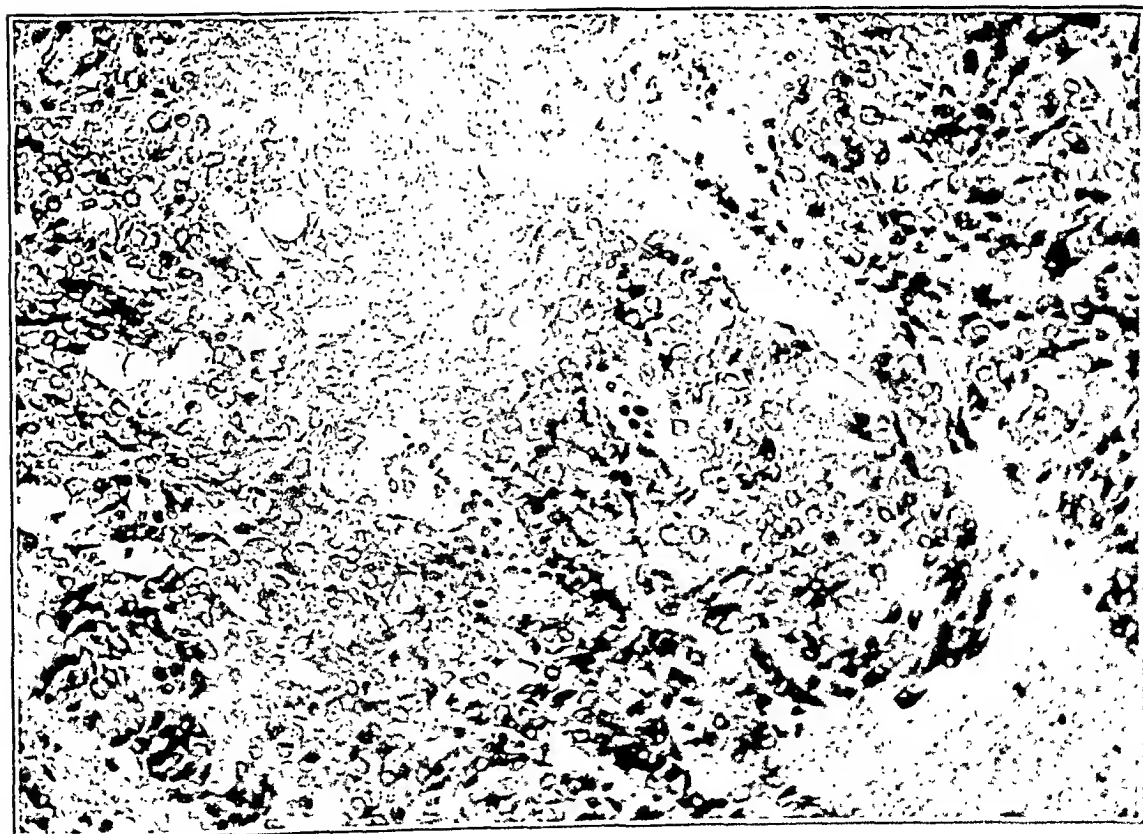
PLATE 132

FIG. 5. Area of peculiar small spindle cells resembling condensed pseudo-syncytial mesenchyma. Malignant course suggested on basis of such area.

FIG. 6. Diffuse recurrent tumor. Growth in sheets of pale syncytial cells resembling an epithelial or a diffuse endothelial tumor.



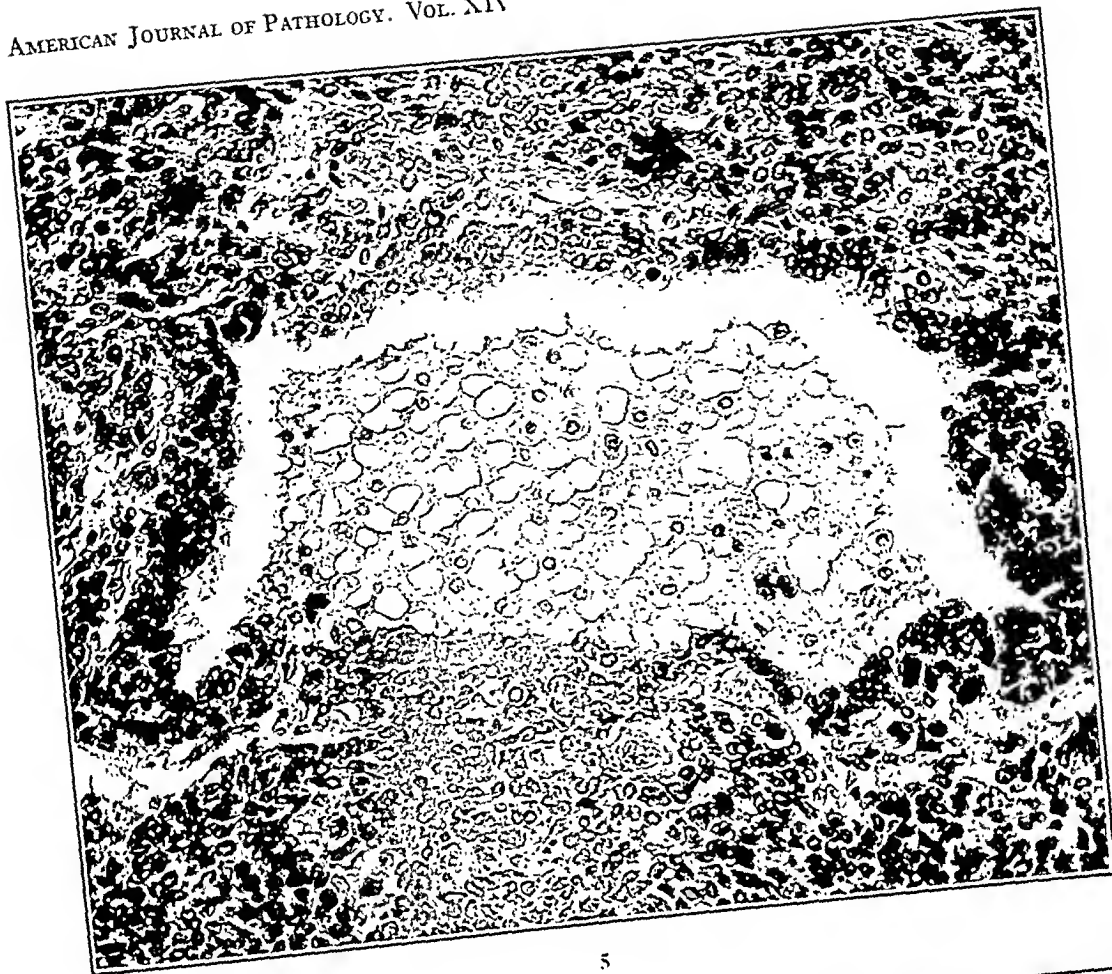
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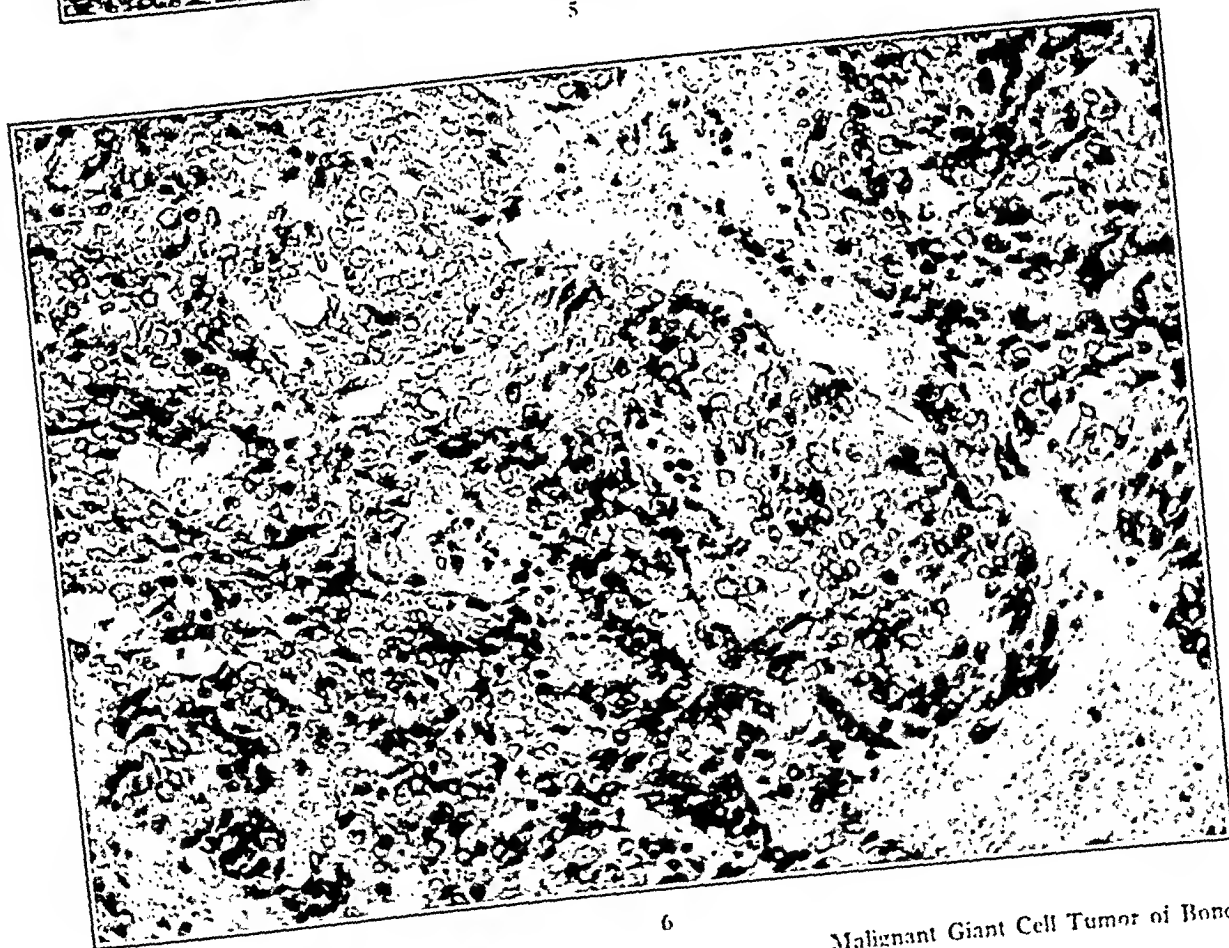
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PLATE 132

- FIG. 5. Area of peculiar small spindle cells resembling condensed pseudo-syncytial mesenchyma. Malignant course suggested on basis of such area.
- FIG. 6. Diffuse recurrent tumor. Growth in sheets of pale syncytial cells resembling an epithelial or a diffuse endothelial tumor.



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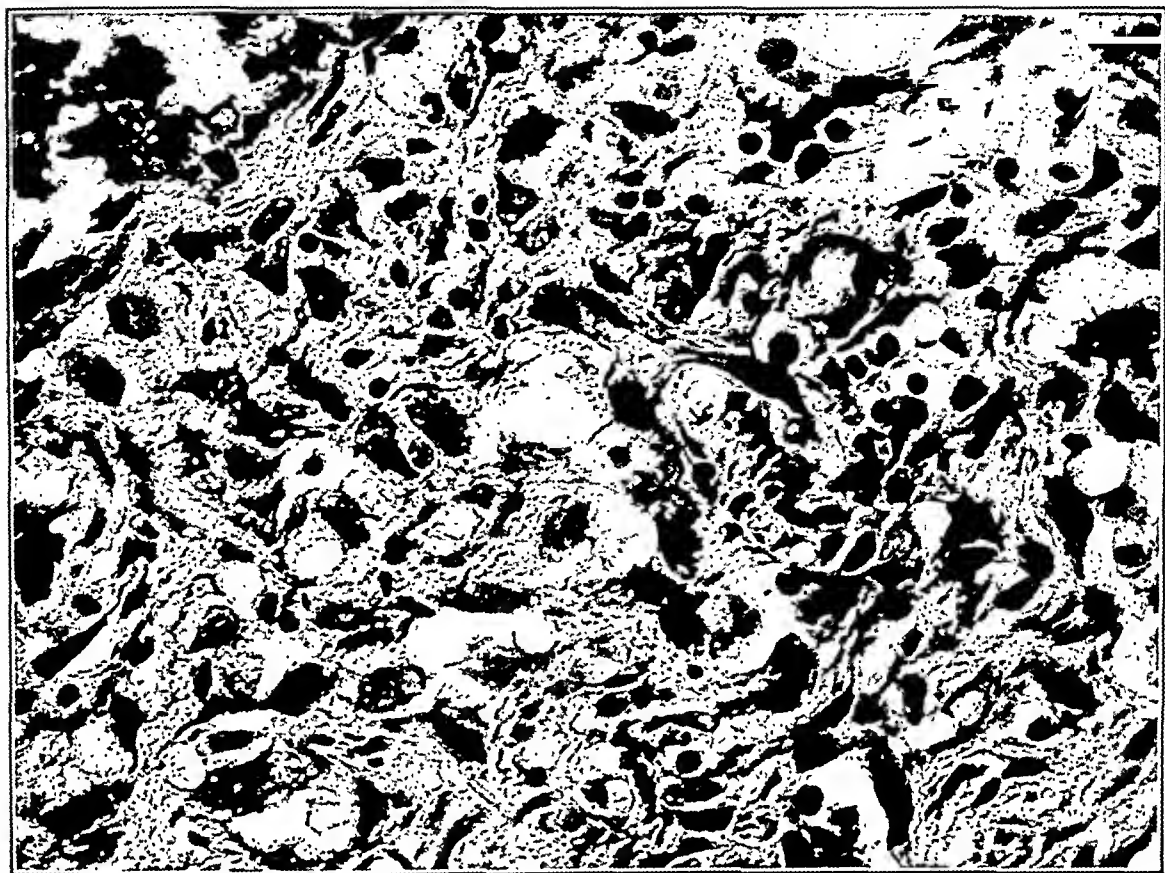
Malignant Giant Cell Tumor of Bone

Stewart, Coley and Farrow

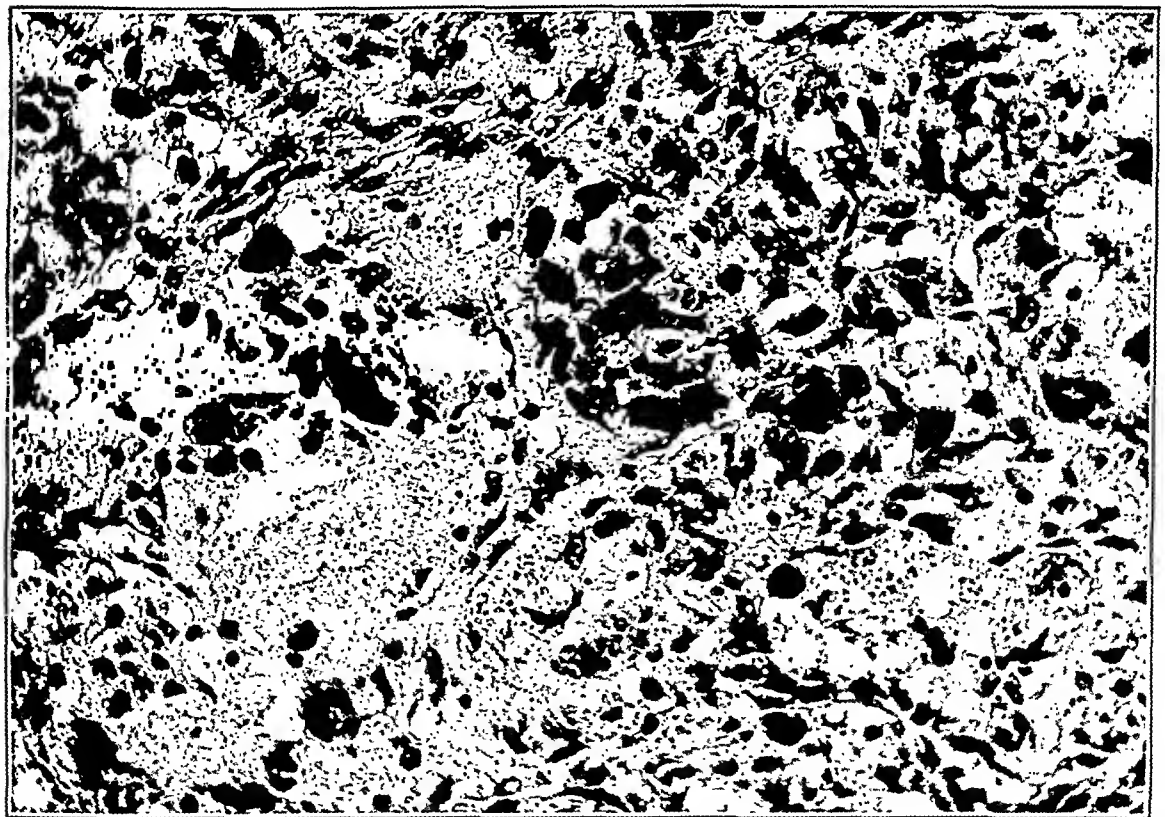
PLATE 133

FIG. 7. Marked angioblastic characteristics in the recurrence of a benign giant cell tumor.

FIG. 8. Malignant giant cell tumor. Distinct angioblastic characteristics.



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8

BLOOD PLASMA PROTEINS AS INFLUENCED BY LIVER INJURY INDUCED BY CARBON TETRACHLORIDE AND GUM ACACIA *

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and Dentistry, Rochester, N. Y.)*

INTRODUCTION

Although the changes in the blood plasma proteins following liver injury are variable and often only transient, a study of the prolonged effects of injurious agents might be expected to throw some light on the problem of plasma protein production. Administration of carbon tetrachloride to dogs has been shown to produce some impairment of liver function as evidenced by a temporary decrease in fibrinogen concentration,¹ and marked anatomical changes of the nature of cirrhosis have been produced by some workers^{2,3} by prolonged administration of this substance. In such animals there have been clinical evidences of liver insufficiency.

By giving carbon tetrachloride to dogs over periods of many months we have been able to produce slight to moderate cirrhotic changes in the liver. A study of the plasma proteins in such animals has shown slight diminution in the total plasma protein concentration with a distinct decrease in the albumin fraction.

We⁴ have recently reported that intravenous injection of gum acacia solution causes lowering of the fibrinogen and total circulating protein, incident to the deposition of acacia in the liver cells. Since both carbon tetrachloride and acacia can be given to dogs over periods of several weeks without marked changes in their clinical conditions, it was of interest to combine the use of these substances † in the same animals, in order to ascertain whether there would be evidence of added injury due to their combined effects, or, possibly, whether acacia might protect liver cells from damage by carbon tetrachloride. The data to be presented indicate that this combination causes a more marked decrease in fibrinogen and total plasma protein concentration than that which has been obtained with gum acacia alone.

* Received for publication May 5, 1938.

† We are indebted to Eli Lilly and Company for valuable materials used in these experiments.

METHODS

Dogs were used for all experiments. Two dogs were studied which received carbon tetrachloride alone. Three dogs were given carbon tetrachloride and gum acacia. Carbon tetrachloride, both C.P. and commercial grades, was given in varying doses by stomach tube. Sufficient water was added in order to diminish as far as possible any local irritative effects. Small amounts of the drug, 2 to 5 cc. daily, were given at first and this dosage was increased as indicated in Tables I and II.

Gum acacia (Lilly, "without sodium chloride") was made up in Locke's solution (minus calcium chloride) to concentrations of 6 or 12 per cent, and the desired amount of this solution was injected intravenously.

Blood for analysis was obtained by jugular puncture and added to measured amounts of 1.4 per cent sodium oxalate solution in hematocrit tubes. Appropriate dilution factors for this procedure were introduced into the various formulas. Total nitrogen determinations were made in duplicate and triplicate by a modification of Goebel's method, as described by Peters and Van Slyke.⁵ Occasional plasma non-protein nitrogen determinations showed no increase in this substance, therefore an average amount, 20 mg., was subtracted from the total plasma nitrogen before multiplying by 6.25 in order to obtain the plasma protein concentration. Fibrinogen was estimated by using a modification of the Jones-Smith method as described by us in a recent publication.⁴ Albumin and globulin were determined by Howe's method as described by Peters and Van Slyke⁵ using 22 per cent sodium sulfate at 37° C.

Dogs receiving carbon tetrachloride were fed various diets. No attempt was made to control the carbohydrate content of the diets, and only at intervals was the protein content measured. Dogs receiving carbon tetrachloride and gum acacia were given diets consisting wholly of cooked meat in order to obtain as great a response to liver damage as possible.²

EXPERIMENTAL OBSERVATIONS

Dogs Receiving Carbon Tetrachloride Alone: Table I (Dog 35-35) shows an 8 months interval in the record of a dog that was

TABLE I
Carbon Tetrachloride Given by Mouth (Dog 35-35)

Carbon Tetrachloride Given by Mouth (Dog 35-35)							Carbon tetrachloride given * cc.
Date	Plasma Protein			A/G ratio	Hematocrit red cell %	Weight kg.	
	Concentration gm. %	Albumin gm. %	Globulin gm. %				
Nov. 4	6.66	3.43	3.23	1.1	51.4	16.6	0
11	6.43	2.89	3.54	0.9	44.1	16.6	0
18	7.58	3.64	3.93	0.9	50.8	—	0
25	7.39	3.20	4.19	0.8	42.6	—	0
Dec. 2	7.71	3.21	4.50	0.7	48.8	16.4	20
9	7.58	2.79	4.79	0.6	42.7	15.7	70
16	7.52	2.21	5.31	0.4	45.1	15.9	30
24	7.69	2.47	4.48	0.5	40.9	14.4	115
30	7.01	2.54	4.28	0.6	39.5	16.2	50
Jan. 6	6.82	2.97	3.94	0.6	40.9	16.5	0
13	7.52	3.36	4.29	0.8	38.8	17.5	9
20	7.26	3.02	4.50	0.7	41.2	17.6	52
27	6.68	2.76	4.12	0.7	42.6	17.5	130
Feb. 3	6.94	3.00	4.47	0.7	43.4	16.9	490
10	7.46	2.91	4.51	0.7	42.9	17.6	875
17	7.41	2.83	4.52	0.7	42.2	18.6	175
29	7.35	2.65	3.93	0.7	46.7	17.1	100
Mar. 5	6.68	2.77	4.47	0.7	46.7	17.2	125
12	6.69	2.23	4.51	0.6	48.4	16.6	50
19	5.96	1.79	4.51	0.6	48.6	16.5	100
26	5.47	1.79	4.52	0.7	40.7	16.6	50
Apr. 2	6.46	2.27	4.03	0.7	43.9	16.1	0
9	6.72	1.96	4.03	0.6	39.6	16.6	0
16	6.98	1.89	3.92	0.5	36.1	—	100
23	7.75	1.72	3.73	0.5	39.2	16.0	280
30	7.60	2.20	3.68	0.5	42.6	—	630
May 7	6.89	1.72	4.24	0.5	43.9	16.2	900
14	8.02	2.58	4.44	0.4	48.3	—	230
21	7.78	2.41	5.02	0.4	40.4	—	50
June 22	7.39	2.41	5.86	0.3	40.2	16.0	150
29	7.39	2.41	5.88	0.5	42.9	16.4	
July 13	7.39	2.41	5.00	0.5	45.8	16.6	

* Figures represent amount given since preceding date.

given carbon tetrachloride by mouth. There was a control period from November 4th to December 9th during which time weekly plasma protein determinations were done. It will be noted that there is little fluctuation in the plasma protein concentration, and only at one period of 2 weeks in the 4th month (April 2nd and 9th) was there any diminution. Otherwise the plasma protein concentration ranged within the control period (November 4th to December 9th) limits, with the exception of one high reading on June 22nd. The albumin concentration, on the other hand, shows a gradual decline reaching its lowest point on May 14th, when it was down to 1.72 gm. per cent, about half of the control concentration. In spite of massive doses of carbon tetrachloride following this period the albumin concentration instead of diminishing further rose appreciably. During a period of a week (January 20th) at which time no carbon tetrachloride was given, the albumin concentration rose to 3.36 gm. per cent from a former average of 2.5 to 3 gm. per cent. During the other period when carbon tetrachloride was omitted for 2 weeks (April 16th and 23rd) there was no rise in the albumin.

The globulin concentration shows changes of interest. During the control period there was an increase in globulin concentration which cannot be accounted for. The diet of the animal had not been changed during this period. The rise in globulin concentration along with the total plasma protein concentration in May and June is also of interest, but no adequate explanation can be offered. During this interval large doses of carbon tetrachloride were given. There was a similar rise of short duration during the 2 weeks immediately after carbon tetrachloride was first started.

In summarizing the course of this animal the depression in plasma albumin appears to be the most significant effect of the carbon tetrachloride administration.

Table II shows the reaction of Dog 35-48 to carbon tetrachloride by mouth for an 8 months period. After administration of the drug for 3½ months the total plasma protein began a gradual decline, and for a period of 4 months thereafter it remained below 6 gm. per cent. Its lowest point, 5.04 gm. per cent, was reached after large amounts, 200 to 525 cc. per week of carbon tetrachloride, had been given. A gradual fall over the whole period is noted in the albumin/globulin ratio due primarily to decrease in

TABLE II
Carbon Tetrachloride Given by Mouth (Dog 35-48)

TABLE II Carbon Tetrachloride Given by Mouth (Dog 35-48)							
Date	Plasma Protein			A/G ratio	Hematocrit red cell %	Weight kg.	Carbon tetrachloride given cc.
	Albumin		Globulin				
	Concentration gm. %	gm. %	gm. %				
Nov. 4	6.37	4.13	2.24	1.8	53.7	16.2	0
11	6.52	3.94	2.58	1.5	51.8	15.7	0
18	6.16	4.16	1.99	2.1	50.8	—	0
25	6.33	4.28	2.31	1.9	48.5	—	0
Dec. 2	6.35	4.52	1.83	2.5	49.2	16.7	20
9	6.78	4.77	2.01	2.2	48.2	16.6	32
16	6.87	4.69	2.18	2.4	49.5	17.4	47
30	6.28	4.26	2.01	2.2	50.6	17.6	25
Jan. 6	6.18	4.14	2.04	2.1	45.9	17.8	30
13	5.99	4.05	1.94	2.0	46.9	18.6	35
20	6.51	4.39	2.39	2.1	51.8	19.5	35
27	6.46	3.88	2.63	1.8	49.7	19.6	35
Feb. 3	6.26	3.54	2.92	1.5	50.2	19.4	25
10	6.38	3.77	2.64	1.5	51.1	19.7	0
17	6.43	3.74	2.48	1.2	50.0	20.2	45
21	6.26	3.96	2.34	1.4	47.9	19.6	45
Mar. 5	6.32	3.82	2.45	1.6	51.9	18.6	45
12	5.84	3.81	2.39	1.6	49.7	19.1	25
19	5.48	3.93	2.34	1.6	54.5	19.2	230
26	6.03	3.71	2.45	1.6	51.4	19.1	90
Apr. 2	5.48	3.22	2.26	1.7	49.2	18.9	185
9	5.71	2.90	2.81	1.1	54.4	18.5	525
16	5.49	3.00	2.48	1.2	50.9	18.0	200
23	5.71	2.77	2.58	1.2	50.6	17.3	350
30	5.04	2.78	2.81	1.1	46.2	17.0	150
May 7	5.55	2.65	2.64	1.1	48.5	—	50
14	5.99	2.54	2.71	1.0	45.8	—	40
21	5.79	2.59	3.06	0.9	40.8	—	40
22	5.51	2.59	2.50	1.0	49.3	17.1	40
29	5.51	3.40	2.59	1.3	49.4	—	—
July 13	—	3.20	2.32	1.2	47.3	16.8	—
		3.18	—	1.4	—	—	—
		—	2.59	—	—	—	—
		—	2.59	—	—	—	—
		—	2.32	—	—	—	—
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* Figures represent amount given since preceding date.

albumin but accompanied by a slight increase in globulin. The red cell percentage showed a tendency to fall during the periods when the total plasma protein was brought to its lowest levels.

Dog Receiving Gum Acacia Alone: Chart 1 shows the reaction

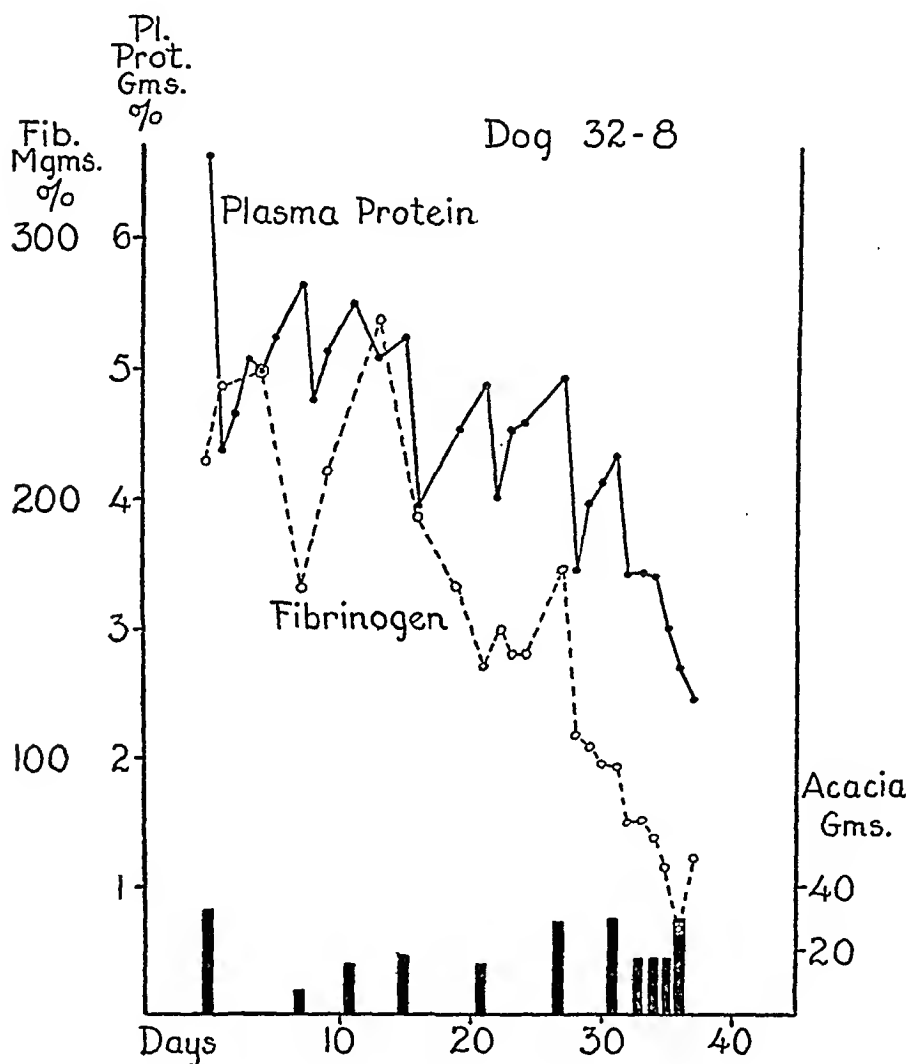


Chart 1. Repeated injections of gum acacia solution.

of a normal dog (32-8) to repeated injections of acacia alone. This dog received a total of 235 gm. of acacia, about 15 gm. per kilo, over a period of 36 days. The plasma protein was gradually reduced to 2.5 gm. per cent and the fibrinogen to 60 mg. per cent. The reaction of this animal is similar to that of other animals that have been studied under the same circumstances.

Dogs Receiving Carbon Tetrachloride and Gum Acacia: Dog 36-189 (Chart 2) received 122 gm. of acacia, a total of 11 gm. per kilo, and 355 cc. of carbon tetrachloride in doses varying from less than 1 to 4.5 cc. per kilo over a period of 38 days. The animal showed a definite fall in plasma protein which was depressed to 2.3 gm. per cent. The fibrinogen showed marked fluctuation after an abrupt fall to below 50 mg. per cent.

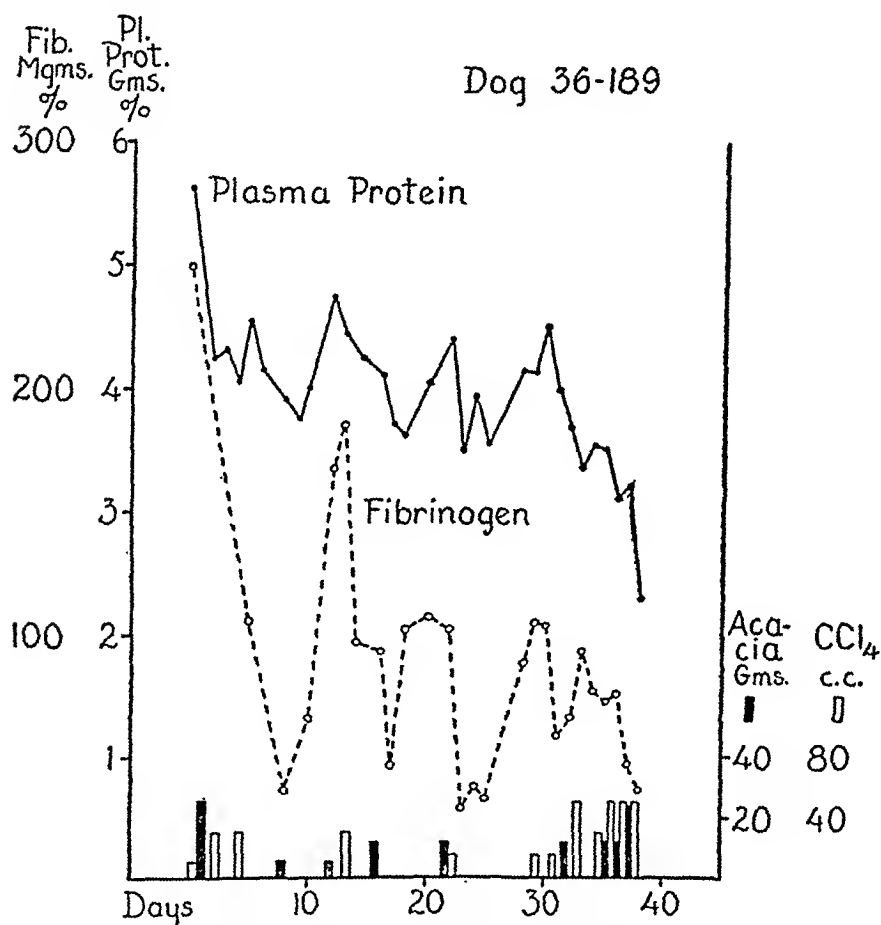


Chart 2. Repeated injection of gum acacia plus carbon tetrachloride orally.

Dog 36-187 (Chart 3) received 91 gm. of acacia, a total of 8 gm. per kilo, and 370 cc. of carbon tetrachloride in doses varying from less than 1 to 4.5 cc. per kilo. In 25 days the plasma protein was brought down to 2.5 gm. per cent and the fibrinogen to below 50 mg. per cent.

Dog 35-145 (Chart 4) received 211 gm. of acacia, representing 12 gm. per kilo, over a period of 78 days. During the first 30 days

when 79 gm. of acacia, or 4.6 gm. per kilo, supplemented by 350 cc. of carbon tetrachloride in doses of 3 and 4.4 cc. per kilo were

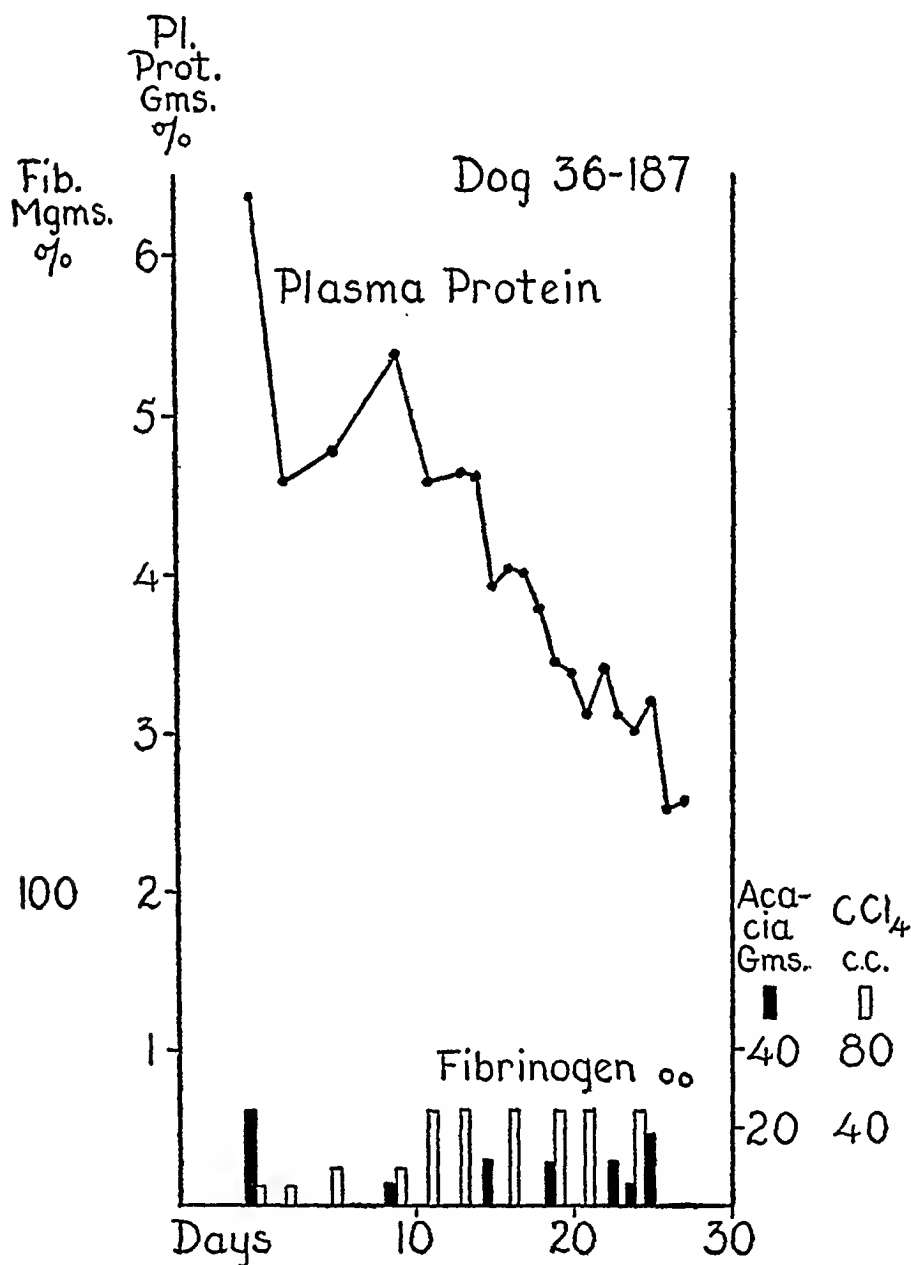


Chart 3. Repeated injection of gum acacia plus carbon tetrachloride orally.

given, the plasma protein was depressed to 3.5 gm. per cent and the fibrinogen to below 100 mg. per cent. Following this the proteins were maintained at a low level by injection of acacia alone.

Although close comparison of these experiments is not possible, it is apparent that combinations of acacia and carbon tetrachloride depress the plasma protein more quickly and to a greater extent than does the administration of acacia alone.

CLINICAL HISTORIES

Dog 35-35: A male shepherd mongrel, vaccinated against distemper. The dog was put under observation on Nov. 4, 1935, and

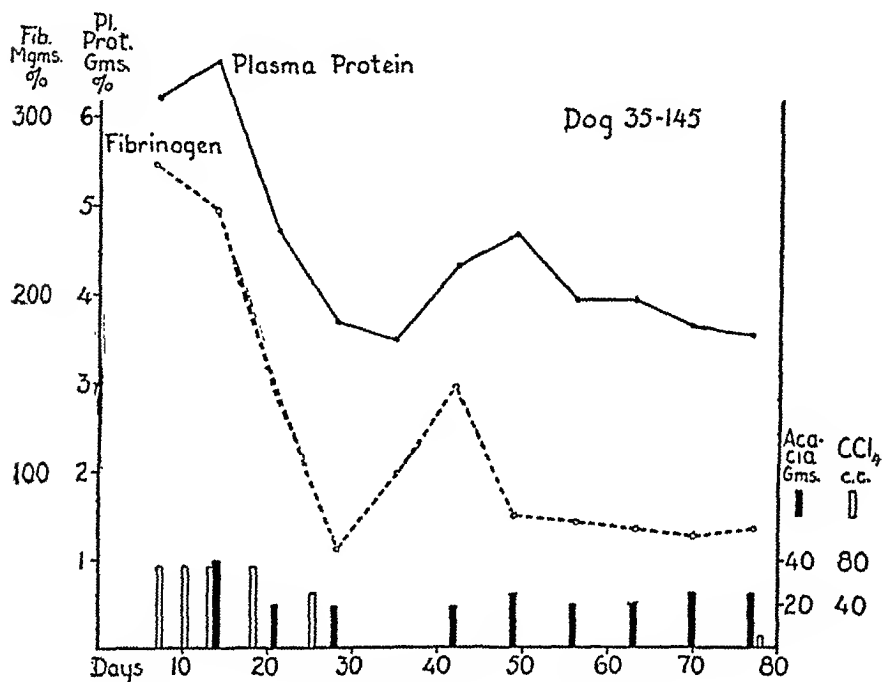


Chart 4. Carbon tetrachloride orally and repeated injection of gum acacia.

on December 4th oral administration of carbon tetrachloride was started, and varying doses of the drug were given for a period of almost a year, until Nov. 11, 1936.

On April 20, 1936, a biopsy of the liver was taken. The liver at operation had a pale yellowish brown color and a very finely granular surface. No gross diagnosis of cirrhosis could be made. Microscopically, however, there was a slight increase in periportal connective tissue, and in some areas, especially near the surface, there was slight distortion of lobulation, the lobules appearing small and irregular. Liver cells about portal areas showed marked fatty changes, containing huge fat vacuoles. About the central areas the liver cells appeared granular but intact and rather pale.

They contained numerous tiny fat droplets. There was no evidence of necrosis.

Biopsy Diagnosis: Fatty liver with early cirrhosis.

Over the entire period the weight remained fairly constant. There was almost constant bilirubinemia which was more marked following larger doses of carbon tetrachloride. No attempt was made to control the diet as to protein content. On Dec. 8, 1936, 4 weeks after the last administration of carbon tetrachloride, another biopsy of the liver was taken. Following this operation the animal did well for 3 days. On the morning of the 5th day it was found dead in its cage. The biopsy section and the postmortem appearance of the liver had essentially the same microscopic appearance.

Autopsy *: The spleen was enlarged, red and tense and on section showed a deep red, rather shiny cut surface.

The liver was large and rather firm. The surface had a finely granular appearance with occasional areas where there was the suggestion of a lobulated pigskin appearance. On section lobulation was prominent and in some places appeared distorted. The portal areas appeared as pale, slightly depressed, moderately wide, irregular gray bands surrounding reddish brown and slightly elevated, small central areas. In gross the liver showed an early cirrhosis.

Microscopic Examination: The liver (Fig. 1) shows moderate increase in portal connective tissue, most of which is delicate. Scattered between fibrous strands are large phagocytic cells which contain yellow granular pigment and large clear vacuoles. There are occasional small islands of liver cells surrounded by connective tissue and in such areas normal architecture is distorted. Also included between connective tissue fibers are occasional, apparently isolated liver cells. Most of the central areas show slight atrophy and numerous small fatty droplets in the liver cells.

Anatomical Diagnoses: Early cirrhosis of liver; congestion of spleen.

Dog 35-48: A young mongrel weighing 16.2 kg. vaccinated against distemper. On Dec. 4, 1935, oral carbon tetrachloride administration was started and this was continued until Nov. 21,

* Routine complete autopsy was done on all animals. Only those organs showing pathological changes are described. All of the viscera, the lymphatic and vascular systems and the bone marrow were examined both grossly and microscopically.

1936 in varying amounts. The dosages employed in this animal were, as can be seen by Table II, considerably smaller than those given the previous dog. At intervals when relatively small doses of carbon tetrachloride were given there was no bilirubinemia, but for the most part there was a moderate degree. During the first 4 months there was a gradual increase in weight of 3 to 4 kg., but from this point to the end of the experiment the weight gradually diminished to about the original level. No attempt was made to control the diet as to protein intake.

A biopsy of the liver was taken on June 1, 1936. Except for pallor, the liver at the time of biopsy appeared grossly normal. Microscopically there was very slight increase in portal connective tissue. Small foci of chronic inflammatory cells were scattered about in portal areas. Liver cells about portal areas contained occasional small fat droplets. Cells in central areas appeared relatively unaltered.

Biopsy Diagnosis: Slight fatty change of liver with slight increase in periportal connective tissue.

On Nov. 21, 1936, carbon tetrachloride was discontinued. At this time the animal was in apparently good condition. The dog died following an operation in December, 1937, more than a year later. At autopsy the liver appear grossly and microscopically normal and no other pertinent abnormalities were noted.

Dog 36-187: An old mongrel hound weighing 11.5 kg. Oral carbon tetrachloride administration was started on Sept. 18, 1936. This was continued for 6 months until the start of the present experiments on March 14, 1937, when in addition to oral doses of carbon tetrachloride, the animal was given frequent injections of 6 per cent gum acacia solution (Chart 3). It was given a diet consisting of 200 gm. of cooked meat. There was slight to moderate bilirubinemia present at all times when the plasma was examined. The animal lost about a pound in weight throughout this latter period. It was found dead in the cage April 10, 1937, and an autopsy was performed. The last 3 days prior to death bleeding from needle puncture wounds in the neck following blood withdrawal had been difficult to stop; it was sometimes necessary to apply pressure for as long as 20 to 30 minutes before bleeding was controlled.

Autopsy: There was no edema or jaundice. The peritoneal cavity was filled with blood-tinged fluid and no clots were present. The spleen was enlarged and tense. There were numerous raised nodules (from 1 to 3 mm. in diameter) bulging above the surface. There was a laceration in the capsule over one of these nodules. The cut surface of the spleen was of a dark purplish red color. The nodules were not sharply demarcated from the rest of the parenchyma but seemed to have a slightly darker color.

The liver had a uniformly dull yellowish brown color with an irregular fine nodular and granular surface. Definite nodules were especially notable along the rounded margins. The organ cut with definite resistance. The cut section showed irregular lobulation with peripheral lobular areas which in many places were wide and quite conspicuous, having a pale waxy gray cast. In some regions coalescence of these foci produced a nodular appearance.

Microscopic Examination: In the spleen malpighian corpuscles are scanty and inconspicuous. The pulp is extremely cellular, being stuffed with hematopoietic elements. In the leukocyte series, myelocytes predominate, numerous mitotic figures being present in them. Many nucleated red cells in all stages of development are present and also mature and immature megakaryocytes. There are heavy hemosiderin deposits throughout the pulp. Such an appearance is not infrequently encountered in old dogs. Many large, clear, finely vacuolated phagocytic cells (gum acacia) are also scattered about which do not contain pigment. These vacuoles do not stain for fat.

The liver (Fig. 2) shows no appreciable increase in portal connective tissue. There is marked contrast between central and periportal regions. In the central areas the liver cells are large and clear with deeply staining compressed nuclei. Some of these cells have a granular appearance due to numerous small clear vacuoles in the cytoplasm. Some smaller clear cells with small nuclei are present. Fat stains show the cells which appear most transparent to be filled with solid masses of fat. The granular cells show occasional fat droplets but for the most part are relatively free of fat. About the portal areas liver cells appear to be flattened and elongated and they are slightly larger than normal liver cells. They contain numerous small clear vacuoles, scattered uniformly throughout the cytoplasm. These do not stain for fat

and are thought to be gum acacia. Most of the Kupffer cells are thin and spindle shaped; a few of them, however, show an increased amount of clear granular cytoplasm.

The kidneys show slight thickening of all glomerular capsules. There are scattered small foci of lymphocytes in the cortex. A few interstitial phagocytes have small fat vacuoles in the cytoplasm. Very infrequent small fat droplets are noted in epithelial cells of the collecting tubules. One glomerular tuft in two sections studied shows vacuolization of endothelial cells composing about half of the tuft. These cells are large and pale. The bone marrow shows essentially normal marrow elements. Infrequent large cells showing the characteristic vacuolization associated with gum acacia are present.

Anatomical Diagnoses: Rupture of spleen; hemoperitoneum; infiltration of acacia in liver cells; fatty degeneration of liver cells in central areas; slight deposition of acacia in spleen; extramedullary hematopoiesis, spleen; fatty degeneration of renal collecting tubules.

Dog 32-8: The detailed clinical history of this animal has appeared in a previous publication.⁴ In brief, it was a normal dog which had received frequent injections of gum acacia solution over a period of a month. At autopsy (following gas anesthesia) the liver showed marked infiltration of acacia into the liver cells. There was slight infiltration of acacia into the splenic reticulum.

Dog 36-189: A young female mongrel weighing 12 kg. Small doses of carbon tetrachloride were started on Sept. 18, 1936, and continued until Dec. 18, 1936. The dog was not used further until April 12, 1937, when intravenous injections of 12 per cent gum acacia solution and oral doses of carbon tetrachloride were started (Chart 2). The experiment was terminated by gas anesthesia on May 21, 1937. Bilirubinemia was present to a moderate degree throughout and the dog lost 2 kg. of weight during the period. It had received a daily diet of 200 gm. of cooked meat.

Autopsy: There was no edema or jaundice. No immediate clots formed from blood released in the cavities during the autopsy, but after several hours a few small pale red clots formed. The spleen weighed 90 gm. and was of rubbery consistence. On the surface

of the organ were large, grayish purple, slightly raised nodulations measuring from 1 to 3 cm. in diameter, which on cut section showed no definite demarcation from the grayish red surface elsewhere but which gradually blended off into a paler gray color near the surface. There was some tarry material in the colon.

The liver weighed 435 gm. The surface of the organ was slightly granular. The margins showed some irregular larger nodules. It was firmer than normal. Cut sections were dark red with distinct and slightly irregular lobulations. The central areas were a distinct deep red. The peripheral zones were pale and sharply demarcated. Retroperitoneal and mesenteric lymph nodes were moderately enlarged.

Microscopic Examination: There are a few small foci in the lungs where the alveolar walls and perivascular fibrous tissue contain occasional large clear cells. Infrequent large clear foamy cells lie free in alveoli.

The spleen shows marked changes. The malpighian bodies are large and stain deeply as contrasted with the pale pulp. In the center of the malpighian bodies are pale cells which contain mitotic figures. Cells lining sinusoids are swollen and have clear cytoplasm with small compressed pyknotic nuclei. Many large cells scattered throughout the pulp have a clear vacuolated appearance. These vacuoles do not stain for fat. Occasional groups of large clear cells are noted between dense connective tissue fibers in the capsule.

The liver shows no appreciable increase in portal connective tissue. The contrast between central and portal regions is marked. In the central areas are characteristic pale vacuolated liver cells with compressed nuclei. Fat stains show moderate sized fat droplets in some of these cells but many vacuoles do not stain for fat. Polygonal cells surrounding the portal areas show numerous small pale vacuoles (gum acacia), but the cytoplasm of these cells does not on the whole have an appreciable degree of pallor. Kupffer cells in portal areas appear compressed, but are otherwise uninvolved. In central areas occasional flat Kupffer cells are noted.

The kidneys show vacuolization and swelling of epithelial cells lining many of the loops of Henle. In the interstices of these tubules are many large pale vacuolated cells. Many epithelial

cells lining collecting tubules show the same appearance. Fat stains show the vacuoles to be composed of fatty droplets. The glomeruli are essentially negative. In the bladder there are occasional groups of pale vacuolated cells in the submucosa between fibrous tissue bundles. In one large lymph node the interstitial tissue and peripheral and medullary sinuses are stuffed with characteristic large foamy cells. Germinal centers are distinct. Sections of other nodes show fewer clear cells. The bone marrow is not remarkable, save for very infrequent large foamy cells.

Anatomical Diagnoses: Infiltration of acacia into liver cells and reticular cells of spleen; moderate fatty degeneration of liver cells in central areas; fatty degeneration of epithelium of renal collecting tubules and loops of Henle; phagocytic cells containing acacia in spleen, lungs, submucosa of urinary bladder and lymph nodes; lymphatic hyperplasia of spleen.

Dog 35-145: A mongrel hound weighing 20 kg., vaccinated against distemper. On Aug. 26, 1936, oral administration of carbon tetrachloride was started and continued until Nov. 11, 1936. Nothing more was done to the animal until March 14, 1937, when carbon tetrachloride administration was resumed. During this interval, which ended on June 8, 1937, the dog lost 2 kg. in weight. On June 8 administration of 12 per cent acacia was started and was continued until the death of the animal on Aug. 16, 1937. Chart 4 shows the plasma protein picture during this interval. There was bilirubinemia throughout and there was a period of about a month from June 15 to July 13 when part of the diet was refused. The diet consisted of 300 gm. of cooked meat daily. After this period, however, the clinical condition was excellent until the day before death, when a short time after administration of a small dose (5 cc.) of carbon tetrachloride the dog had a convulsion. It was listless the remainder of that day and was found dead in the cage the following morning.

Autopsy: There was slight icterus of the sclerae and subcutaneous tissues. 30 to 40 cc. of bloody fluid were present in the peritoneal cavity. The heart showed two large areas of subendocardial hemorrhage in the left ventricular wall. The lungs showed moderate congestion and edema. The spleen was soft and mushy. The pulp scraped away readily. The lower part of the colon and

the rectum contained blood stained feces. No gross ulceration was noted.

The liver weighed 610 gm. It was deep red in color with multiple yellowish projections from the surface, 1 to 5 mm. in diameter. These were slightly firmer than the surrounding tissue which was quite soft. The cut surface showed a granular appearance. The lobules were prominent. Yellowish nodules similar to those seen on the surface were scattered throughout the parenchyma. There seemed to be considerable autolysis.

Microscopic Examination: The spleen shows marked autolysis but some mononuclear cells with typical foamy appearance are present in the sinusoids. The pancreas shows marked autolysis and some hemorrhage into the peripancreatic fat. The liver shows marked autolysis. In the shadowy structure, however, islands which have the general configuration of lobules are seen surrounded by wide striated bands which seem to have been connective tissue. Occasional islands of liver cells show considerably less autolysis and in the polygonal cells in these areas are seen numerous characteristic small clear vacuoles resembling those associated with acacia in other animals. The kidneys show marked autolysis but the epithelium of some of the loops of Henle contains large and small clear vacuoles which stain for fat.

Anatomical Diagnoses: Moderate cirrhosis of liver with infiltration of acacia in liver cells; bloody fluid in peritoneal cavity; blood in feces; hemorrhages beneath the epicardium and about the pancreas; fatty degeneration of renal epithelium (loops of Henle).

DISCUSSION

The clinical condition of dogs receiving gum acacia solution is worthy of note, for these animals whether they receive gum acacia alone, or combined with carbon tetrachloride, show remarkably little change in their general appearance and activity. In spite of reduction of plasma protein concentration well below the edema level they had no edema. They were active and for the most part consumed their diets with avidity, although most of them showed a slight loss in weight. One outstanding feature should be stressed. When samples of blood were taken by jugular puncture in later periods there was frequently prolonged bleeding from the needle wound for as long as 30 minutes. This is ascribed to the low

fibrinogen concentration, but the possibility that it might also be related to a diminution of prothrombin associated with the liver injury must also be considered. Smith, Warner and Brinkhous⁶ have recently described such a state in dogs whose livers were injured with chloroform.

The 2 dogs receiving carbon tetrachloride alone showed an increase in portal connective tissue with well marked fatty change of hepatic cells. These dogs showed slight decrease in plasma protein concentration which appeared to be due chiefly to loss of albumin. These findings agree with those of Bollman* and they certainly appear to be associated with liver injury.

There is some evidence brought out by different types of hepatic damage indicating a separate production of the different components (albumin, globulin, fibrinogen) of plasma protein. Dogs receiving gum acacia over a long period of time have shown more marked diminution of globulin than of albumin.⁴ This cannot be explained by loss of fibrinogen, which is included in the determination of the globulin, and which amounts to but a small fraction of the total decrease. On the other hand, dogs with livers injured by carbon tetrachloride, or Eck fistula dogs which have received no acacia † exhibit low albumin content. Fibrinogen is of interest in this regard because it is the most labile of the plasma proteins in response to hepatic injury. It is the first to show a decrease following liver damage. The limits of its fluctuation are much wider than those of the other proteins. Thus it would seem that wherever the site of plasma protein production is (and evidence indicates that this occurs largely in the liver) there may be a separate specialized intrahepatic mechanism for the elaboration of each of these basic components of the plasma proteins.

Of the 3 animals that received carbon tetrachloride and gum acacia, only 1 (35-145) showed evidence of increased portal connective tissue at autopsy. All of these animals had received carbon tetrachloride over periods of several months previous to the start of the present experiments, and 2 of them (36-187, 35-145), had been given this substance for several months immediately preceding the beginning of injections with acacia. The changes of the plasma proteins in the individual animals are so similar it would

* Personal communication.

† Unpublished data.

seem that the presence of fibrosis in the liver did not modify the reaction of this particular animal to the procedure. In this group of dogs changes in plasma protein and fibrinogen concentration are somewhat more marked than in dogs receiving acacia alone, and although one cannot rule out completely the possibility that splenic involvement might modify to some extent the plasma protein picture, changes in fibrinogen, which under these conditions are marked, probably cannot be ascribed to anything but hepatic injury, inasmuch as the liver⁷ is thought to be the sole source of fibrinogen.

Dogs receiving gum acacia injections, whether combined with carbon tetrachloride or not, have always shown characteristic fine vacuolization of liver cells. Such vacuoles have been noted whether the animals have received a few injections of acacia or considerable amounts of the gum over relatively long periods of time.^{8, 4} These vacuoles do not stain for fat or glycogen, and although they cannot be identified as acacia by a specific stain, their presence is so constant that there can be little doubt that they represent some form of this material. Acacia has been demonstrated chemically in such livers by Andersch and Gibson,⁸ and by us. In studies to date dogs that have received gum acacia have shown more marked changes in the liver than in any other organ, although there has been slight infiltration of acacia into the splenic pulp. In the animals that have received carbon tetrachloride in addition to gum acacia, the spleen and occasional lymph nodes have shown the characteristic intracellular droplets to a greater degree than have dogs receiving acacia alone. These findings have not been constant, however, and the livers of such animals have also shown the most marked changes. More evidence, therefore, is brought to light that the liver is intimately associated with the production of the plasma proteins.

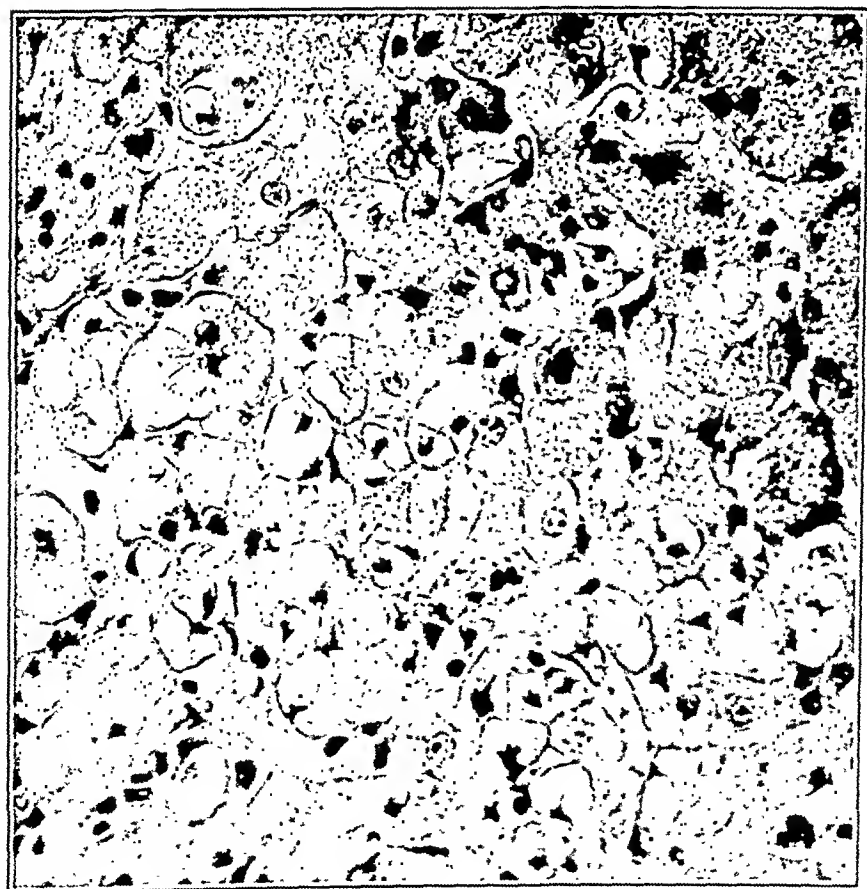
SUMMARY AND CONCLUSIONS

By frequent oral administration of carbon tetrachloride to dogs it has been possible to produce moderate cirrhotic changes in the liver. In such animals the plasma protein concentration falls slightly, and this decrease appears to be due largely to loss of albumin.

The continued administration of gum acacia, combined with



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DESCRIPTION OF PLATE

PLATE 134

- FIG. 1. Microphotograph of a section of liver from a dog (35-35, Table I) that had received carbon tetrachloride orally. Increase in portal connective tissue, irregularity of lobulation, and fatty changes of liver cells about portal areas are shown. Hematoxylin-eosin stain. $\times 100$.
- FIG. 2. Microphotograph of a section of liver from a dog (36-187, Chart 3) that had received carbon tetrachloride and gum acacia. The cells in the upper right corner show vacuolization associated with acacia injection. The cells on the left contain chiefly fat. Hematoxylin-eosin stain. $\times 430$.

A COMPARATIVE MORPHOLOGICAL STUDY OF THE MAMMARY GLAND IN A HIGH AND A LOW TUMOR STRAIN OF MICE *

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In the Little-Murray inbred dilute brown strain of mice 80 per cent of the females with a normal reproductive history develop spontaneous carcinoma of the mammary gland. For this reason the strain can be considered as a high tumor line. In the C57 black strain of mice spontaneous mammary carcinoma appears in less than 1 per cent of the breeding females. This strain can, therefore, be considered as a low tumor line. The tumors are detected when they are large enough to be palpable. At this time microscopic examination usually reveals a well established malignant condition. By sectioning and comparing many mammary glands of the two strains it was thought that it might be possible to detect certain early structural changes and differences which might be considered as steps leading to abnormality. It is the purpose of this paper to describe the results of such an investigation.

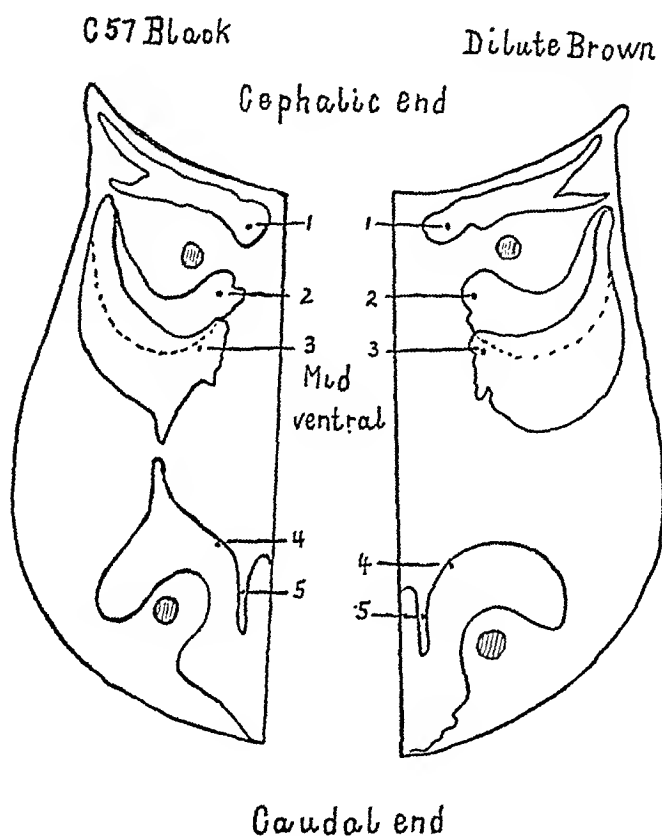
The structure of the mammary gland of normal mice has been studied by several investigators. Among the more recent reports those of Turner and Gomez,¹ and Cole² are the most comprehensive.

Gibson³ made a comparative study of the life history of the mammary glands of two strains of albino mice, one of which was a high, the other a low tumor strain. In the high tumor line she noted different anomalies in the nipple development, neoplasms developing in zones of chronic cystic mastitis, and the fact that the epithelial elements of the gland of the high tumor strain were inclined to metaplasia rather than to atrophy as age advances. Gardner and Strong⁴ studied the mammary glands of virgin females in ten strains of mice differing in susceptibility to spontaneous neoplasms. They found no structural factor in the development of the mammary gland associated with the intrinsic hereditary predisposition to mammary carcinoma in mice.

The present investigation started with the examination of 1

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mals. The distance between the thoracic and inguinal glands in the dilute brown mice is greater than in the black mice. Text-figure 1 is an actual copy of the outlines of the glands of the two strains. While this greater distance was found to be constant in the dilute brown mice, in a few cases the extension of the gland in the black mice was similar to that in the dilute browns.



TEXT-FIG. 1. Diagram of the skins of the inner surface in the two strains of mice showing the position of the mammary glands. The drawing was made by tracing the outlines as they appeared on carefully skinned specimens. The position of the nipples is shown by numbered dots. The intermittent lines show the outlines of the 2nd glands which are overlapped by the 3rd glands. The shaded circles mark the position of the legs.

Observations: The nipples of the 1 week old mice can be located under a dissecting microscope. From each nipple one duct leads to the subcutaneous fat pad where it branches into a few secondary ducts. The subcutaneous fat pads are divided into lobes and lobules, and have a good blood supply. The main duct of the 1st

week old animals, continued at weekly intervals until the 4th week, and at biweekly intervals until the first pregnancy — about 6 to 8 weeks. From this time on the physiological condition of the mammary gland was taken as a basis for comparison rather than the chronological age of the animals, because it is important to know the condition of the females in regard to their reproductive phase at the time the mammary glands of two animals are compared. Without this knowledge the examination would lead to erroneous interpretation.

Pregnancy was timed from the appearance of the vaginal plug. As a routine the animals were separated from the breeding pen to individual pens at the later stages of their pregnancy. They were kept there until the young mice were weaned — 3 to 4 weeks after birth. The date of weaning depends somewhat on the size of the litter and general condition of the offspring. On the average a breeding female has a litter every 2 months.

Technical Procedure: The animals were killed by ether. The skin was cut open on the mid-dorsal line and carefully removed, with the subcutaneous fat pads in normal position. The skin was spread and pinned out on a layer of paraffin hardened on the bottom of a glass dish and flooded with the fixing fluid. A mixture of formaldehyde (10 cc.), 70 per cent alcohol (100 cc.) and acetic acid (5 cc.) was used for fixation. All the glands were carefully studied under a dissecting microscope. All the glands with the nipples from the right side were cut away from the skin surface, dehydrated, cleared and embedded in paraffin. In the earlier stages serial sections were cut at right angles to the skin surface. In the later stages the sections were cut parallel with the skin surface. This way many sections of full surface of the glands were available for examination. Hematoxylin and eosin stains were used.

Mice have 5 nipples on each side — 3 in the thoracic and 2 in the abdomino-inguinal region. Each nipple with its ducts, collaterals and terminal branches is a separate unit and is not in communication with the others. Throughout this work the glands will be designated with numbers from 1 to 5 starting with the one most cephalad.

It was observed that a difference exists between the extension of the fully developed glands of the dilute brown and black ani-

Between the ages of 6 and 8 weeks female mice reach sexual maturity. The changes that take place in the gland after that time are under the influence of the periodic sexual cycles of the animal. At the time of the first ovulation an acceleration of growth occurs and the ducts develop many side branches. The distal ends of some of the side branches terminate in bulb-like enlargements. Microscopically the end bulbs show that they are lined by many layers of cuboidal epithelial cells. Mitotic figures are numerous and signify rapid growth. The stroma around the end bulbs shows slightly increased fibrosis (Fig. 1).

A slight increase in the growth rate and dilatation of the ducts is noticeable every time the animal approaches estrus and is followed by a slight regression. If ovulation is followed by fertilization and pregnancy, development progresses rapidly. The ducts increase in length and many side branches are formed. The subcutaneous fat pads seem to limit the growth of the gland system and the original lobes and lobules of this adipose tissue form the framework of the fully developed gland. If fertilization does not occur there is a slight regression.

In order to be able to observe the possible individual variations within the normal, more than one animal of each of these stages was studied. The glands of 11 dilute brown and 11 C57 black mice were sectioned and examined up to this stage and showed no significant morphological difference.

First Pregnancy: During pregnancy the epithelial elements of the glands gradually enlarge by cell division. An increase in the number of mitotic figures is definitely noticeable at the 4th to 5th days of pregnancy and reaches its peak at about the 11th to 12th days (Fig. 2). This was ascertained by counting the mitotic figures in several cross sections of ducts. No great difference was found in the rate of growth between the dilute brown and the C57 black mice.

During this period many end bulbs are present at the distal ends of the ducts. After the first litter has been raised end bulbs do not occur on the glands during subsequent pregnancies. By further development all the bud-like endings and the terminal twigs dilate and unfold into alveoli.

At 14 to 15 days of pregnancy the glandular system is well developed and mitotic figures are rare. Only at the distal ends

nipple grows cephalad, those of the 2nd, 3rd and 4th nipples grow dorsolaterally, and the main duct of the 5th nipple grows caudad. The primary duct of the 5th nipple has a longer, straight unbranched portion than any of the others.

Microscopically it is shown that at the site of the nipple the epithelium is thickened and the stratum granulosum and germinativum form a bell shaped epithelial cone which projects down into the subcutaneous tissue. In the middle of this the primary duct penetrates at a right angle to the skin surface, turning parallel with the skin surface as soon as it reaches the subcutaneous tissue. The lining of this duct is very compact and consists of low cuboidal epithelial cells. The epithelial cells of the secondary ducts are somewhat taller than those in the main duct. A fibrous envelope surrounding the ducts becomes gradually thinner toward the end distal to the nipple.

When the animals are 2 weeks old the skin at the site of the nipples is more elevated. The sections show that the bell shaped epithelial cone projects somewhat deeper and is surrounded by a sulcus. The ducts are longer and have more collateral branches.

At 3 weeks it is more difficult to detect the area of the nipples because the hair is growing rapidly and covers them. Microscopically sections show that the sulcus surrounding the nipples is deeper, and circular epithelial folds are beginning to form around the nipple. The ducts are considerably longer and have more side branches.

When the glands are fully developed the 2nd and 3rd glands overlap each other and are separated by a thin layer of muscle — the panniculus carnosus. The duct system of the 3rd gland has to penetrate through this muscle layer. This probably is the explanation of the fact that ducts of the 3rd nipple are somewhat slower than the others in development.

At 4 weeks the nipples are elevated above the skin surface and the circular folds are visible. The circular folds give the elasticity to the nipple which can be considerably stretched out by the young mice at the time of nursing. The development of the duct system has progressed further and produced more side branches.

The development progresses steadily up to the age of 6 weeks. This completes the prepubertal period which is characterized by regular progressive growth.

Riddle, Bates and Dykshorn⁶ were able to extract this secretion-stimulating hormone from the anterior pituitaries of beef and named it prolactin. Allen, Gardner and Diddle⁷ were able to induce lactation in ovariectomized monkeys by injecting theelin followed by galactin or prolactin.

First Lactation: The young mice are born on the 19th to 21st day of gestation. The first few days after parturition all the glands are in full function. The alveoli are comparable to a bunch of grapes — they are firm, round and white. Grossly they appear as uniformly distributed, small white granules which surround and obscure the ducts.

Microscopically sections show that the ducts near the nipple are dilated and contain milk. The lumens of the alveoli are large and contain droplets of secretion. The close contact of the epithelial cells with capillaries is evident and erythrocytes are seen in single layers within minute capillaries between the alveoli. Fibrocytes with flattened, deeply staining nuclei follow the course of these capillaries. The glandular parenchyma is in excess and adipose cells serve only to fill in the space left by it. As mentioned previously, the original lobes and lobules of the fat pad supply the framework of the lactating gland (Fig. 4).

The epithelial cells of the alveoli are not uniform but show all phases of activity. In some cells the nucleus is in the middle of the cell and the cytoplasm is homogeneous. In others the cytoplasm appears foamy and contains large protruding droplets. The nuclei have been pushed aside and flattened against the cell walls. Mitotic figures are rare although not entirely absent.

It was noticed after the 7th day of lactation in both the C57 black and the dilute brown animals that if the litter is small, some of the nipples are not suckled. The glands leading to these nipples differ from the nursed glands. The difference is noticeable grossly on the fixed skin. These glands are more yellowish in color as compared with the white, turgid suckled glands. The alveoli are shrunken and the enlarged ducts are plainly visible. Microscopically it is seen that these glands have undergone a certain degree of regression. The amount of adipose tissue is greatly increased, the ducts are distended with milk and the alveoli are decreased in size and number. The cells of the alveoli are irregular but many of them are functioning. Most often it is the first pair of glands

away from the nipple are the ducts still growing. Further development consists of hypertrophy and an increase in size of the individual epithelial cells and the enlargement of the lumens. The ducts end in terminal alveoli which at this stage begin to show secretory activity. This activity starts first in the alveoli proximal to the nipple and progresses gradually distally. First small droplets, later larger vacuoles appear in the cytoplasm. The nucleus is pushed to the periphery away from the lumen. At 17 to 19 days secretory activity is generally well established throughout (Fig. 3). Parallel with the glandular elements there is an intensive development of blood vessels. The developing ducts follow the course of these vessels and capillaries are seen to be in intimate contact with the secreting epithelial cells. The developing glandular system occupies more and more space and the adipose tissue of the fat pads is rapidly diminishing.

The glands of 7 dilute brown and 6 black mice were included in this group. The sections were cut parallel with the skin surface, making the whole extent of the glands available for examination. All the mice were between the age of 2 and 3½ months old.

No structural difference was found between the two strains of mice.

From these observations it is evident that the changes taking place in the mammary gland during pregnancy can be divided into two periods. During the first period, which lasts until the 13th to 14th day of pregnancy, there is an acceleration in mitotic activity which results in a numerical increase of the epithelial cells of the glands. During the second period the epithelial cells increase in size and begin to secrete. The development of the mammary gland during the first part of pregnancy is under the hormonal influence of the ovaries, while during the second part the anterior pituitary hormone stimulates the gland to functional activity.

The experimental work which supports this knowledge was well reviewed by Nelson and Piffner.⁵ These authors supplied further evidence by injecting lutein extract into immature female rats and observed marked development of the mammary gland. When a series of estrin injections was followed by a number of lutein treatments before the hypophyseal extract was employed in castrated or immature females, the glands enlarged and milk was secreted.

pyknotic nucleus fragments. These cells are seen in increasing number as round, pink staining bodies with two or more deeply staining dots (Fig. 7). In some epithelial cells the swollen cytoplasm forms globules which are discharged into the lumen, but the nucleus with a small amount of cytoplasm remains intact. As the process of regression advances it is evident that the alveoli are gradually becoming smaller. On account of this they lose their close contact with the capillaries.

The lack of sufficient blood supply hastens the process of regression. The space between the shrinking alveoli is being filled by adipose cells. Some of these cells seem to develop from the fibroblasts which are in close proximity to the capillaries. In the lactating gland these cells have an elongated, deeply staining nucleus and very little cytoplasm. At the beginning of regression they begin to change. The nucleus becomes rounded and the amount of cytoplasm increases. Gradually fat accumulates in the cytoplasm and the nucleus is pushed to the periphery. During this change the size of the cells increases immensely in all dimensions. The increase in the amount of adipose cells is first noticeable interlobularly and later intralobularly.

Four days after lactation stops the alveoli are much smaller and are very irregular in outline. Some of the alveoli are collapsed and form irregular clumps of cells. In others the cytoplasm is still swollen. There are a few degenerating cells which have pyknotic and fragmenting nuclei. The lumens of several alveoli are still filled with secretion but the globules have disappeared and the secretion is homogeneous. The main ducts proximal to the nipples are still somewhat enlarged. They contain a few degenerated cells and secretion. The amount of adipose tissue is greatly increased.

Six days after the discontinuation of nursing most of the alveoli are collapsed and form irregular groups of cells. The nucleus is small and compact — the cytoplasm is no longer swollen. Several of the cells have pyknotic, a few of them fragmenting, nuclei. The clumps of epithelial cells are surrounded by fibrous connective tissue. After the first lactation period is over the amount of the fibrous connective tissue surrounding the parenchyma is more compact than before. The amount of adipose tissue increases proportionately with the regression of the parenchyma. Most of the adipose cells have attained full size by now, but some of them are

that is not used (16 out of 29 unsuckled nipples), but evidently it might be any of the glands (Figs. 5 and 6).

Cole² noted such regressions before weaning. In his material the 2nd and 3rd thoracic glands were most often neglected.

To ascertain that such regressive changes are really due to disuse, 2 of the nipples on the right side of a mouse lactating for 14 days were closed with celloidin. The animal was killed 5 days later and the glands of the right and left side were compared. The condition of the glands leading to the closed nipples proved that such regression is due to disuse. This emphasizes the importance of killing the animal and examining the condition of all the glands. Taking just part of a gland under anesthesia might lead to erroneous conclusions.

Microscopically little change in the structure of the glands during lactation in the 12 to 17 day period is seen. The development of the nursing young mice depends to a certain degree on the size of the litter. Consequently the mammary glands of a mouse nursing a large litter are still in full function at 3 weeks after parturition, while the glands of one nursing a small litter begin to show regressive changes at that time. The mother is usually separated from her young between the 3rd and 4th week after parturition, but the glands can be kept functioning for a prolonged period if nursing is continued by younger mice. In the glands of 3 of the dilute brown and 2 of the C57 black mice small areas of acute mastitis were observed showing polymorphonuclear leukocytic infiltration. The glands of 6 dilute brown and 6 black mice were included in this examination. They were between the ages of 2½ and 4½ months.

Regression after First Lactation: Regression is a reversed process of the changes that take place during pregnancy. Grossly there is a gradual decrease in the thickness of the gland. As the milk disappears from the alveoli the white granular appearance changes, the ducts become visible and the whole gland becomes yellowish in color. Microscopic sections show that 24 hours after suckling ceases, milk has accumulated in the ducts and alveoli, which become distended. A few epithelial cells are seen lying loose in the lumens. On the 2nd day of regression the distention of the ducts is further increased. The loosely lying epithelial cells undergo degeneration, the cytoplasm becomes swollen and the

not noticeable grossly or under the dissecting microscope. The general development of all the glands was quite uniform. Mitotic figures were rare; the epithelial cells of the alveoli were undergoing hypertrophy and were beginning to secrete. In the abnormal areas the epithelial cells did not show hypertrophy and functional activity but remained small and compact and showed numerous mitotic figures. Some alveoli and the ducts leading to them had no lumens but were composed of solid cords and groups of cells. While most of the cells appeared to be limited by a basement membrane, some of them were beginning to invade the surrounding tissue, become disorganized and show definite signs of carcinoma. At such areas the cells were slightly larger and stained lighter. The nucleus was less compact and the amount of cytoplasm was increased. The cellular density of the surrounding stroma was increased (Figs. 9 and 10).

Dilute brown mouse No. 5430 had been pregnant the second time for 19 days. Macroscopic abnormalities could not be observed. Microscopically uniformly developed glands which were composed of secreting alveoli were seen. An abnormal area was present on the 1st cervical gland and involved a whole small lobule. Secreting cells were absent in this area, but mitotic figures were numerous among the epithelial cells as well as among the fibrous connective tissue cells of the stroma. The arrangement of the epithelial cells was not atypical and the area did not show a carcinomatous structure although physiologically the cells seemed to be out of normal control, persisting in cell multiplication, while in the cells of the surrounding area secretion had started (Figs. 11 and 12).

The changes in the glands of 1 other dilute brown and 5 C57 black animals examined during the second pregnancy were comparable with those described at the first pregnancy and showed no abnormalities. All the mice in this group were between the ages of 4 and 5 months.

Second Lactation: Three of the dilute brown animals examined while suckling their second litter had normal lactating glands. One dilute brown which was lactating 7 days (No. 5636) showed an area of chronic mastitis on the distal part of the 4th gland. The ducts were densely infiltrated with polymorphonuclear leukocytes. The surrounding area showed lymphocytic infiltration.

still in the process of expansion. The epithelial cells of the terminal ducts undergo the same changes during regression as the alveoli. The lumens of the ducts decrease greatly in size, but the epithelial cells lining it retain their orderly arrangement.

On the 9th to 11th days of regression the few alveoli which are still present consist of small, compact, circularly arranged cells containing deeply staining nuclei and very little cytoplasm. They are closely grouped around the ducts and are surrounded by fibroblasts and a large amount of adipose cells. The main ducts proximal to the nipple are still slightly dilated and contain secretion and a few dead cells.

About 11 to 12 days after nursing stops regression is usually complete. The ducts proximal to the nipple have larger lumens than previous to the first lactation. Distal to the nipple the lumens become smaller, finally consisting only of rows of cells in the terminal branches. There are no alveoli. The gland can be now considered a "resting" gland (Fig. 8).

The process of regression is not very uniform. Considerable variation exists between the glands of the same animal, depending on whether the nipple was suckled or not up to the time of weaning.

The examination of the glands of 5 dilute brown and 4 black mice is included in this group. In the glands of 1 dilute brown and 1 C57 black animal small areas of acute mastitis were found. All the mice were between the ages of 3 to 5½ months. No significant structural differences were found between the dilute brown and the C57 black strains of mice.

It was during the period of second pregnancy that some interesting abnormalities were found in the dilute brown strain. These will be described in more detail.

Second Pregnancy: With the beginning of the second pregnancy there is again a period of rapid development of the glandular parenchyma. The rate of growth increases, as during the first pregnancy.

At 7 days pregnancy the changes were similar in both strains to the development taking place during the corresponding period of the first pregnancy.

At 15 days pregnancy in dilute brown No. 4540 abnormal areas were observed in the 3rd gland. The areas were small and were

lobules which was found on the 1st gland contained a few alveoli in which the epithelial cells had undergone metaplasia, changed into stratified squamous cells and filled the lumens with cornified cell débris (Fig. 13). The stroma surrounding this area showed fibrosis and some lymphocytic infiltration. It is probable that these functioning areas persisted since the previous lactation. Two small areas of acute mastitis with dense polymorphonuclear leukocytic infiltration were also observed on this gland. Dilute brown No. 10807 which was pregnant for 9 days had a large abscess surrounded by a fibrous capsule on the 3rd thoracic gland. A small area showing metaplasia and cornification of epithelial cells was found on the 5th gland. In the fibrous stroma which enclosed this area polymorphonuclear leukocytes and lymphocytes were observed. The rest of the glands showed the usual rapid development, which is characteristic at this stage of pregnancy. Two more dilute brown females pregnant for 15 and 19 days respectively showed normal gland structures.

The 3 C57 black animals examined during their third pregnancy did not show any structural abnormality. All these mice were between the age of 7 and 9½ months.

Third Lactation: Dilute brown No. 92675 had been nursing for 7 days. As there were only 2 young in the litter, all the glands were not suckled and showed irregular regression. In the 1st, 3rd and the 5th nipple the primary duct was greatly dilated and was full of milk. The amount of adipose tissue was increased. The alveoli showed much variation in size and activity but most of them were in the process of regression. Several small areas showed chronic mastitis with fibrosis and lymphocytic infiltration. The 2nd and the 5th glands were lactating normally. Another (dilute brown No. 2816) was also suckling 2 young mice only and showed regression, due to disuse in several glands. Similar irregularities and abnormalities that were described in the glands of the previous mouse were found in this gland too. In a 3rd (dilute brown No. 97374) the 1st nipple was infested with mites. They evidently penetrated to a short distance into the lumen of the nipple and were sectioned lying within the lumen. The epithelial lining showed signs of irritation—increased amount of keratinization and lymphocytic infiltration.

One of the C57 black animals examined at this stage had fully

Some of the alveoli were obliterated and the stroma showed fibrosis. Three of the C57 black animals had normal lactating glands.

In 1 of the black animals which was lactating 6 days (No. 10526) the 1st nipple was filled with cellular débris which evidently caused obstruction. The primary duct leading to this nipple was greatly distended while the rest of the gland structure showed regression due to disuse.

It was observed in both strains during this period, as in the period of first lactation, that the glands that were not suckled were undergoing regression. Again the 1st nipple and gland structure leading to it were most often subject to disuse.

All 4 dilute brown and 4 black mice examined while lactating their second litter were between the ages of 4 and 6 months.

Regression and Resting after Second Lactation: Dilute brown No. 5432 was killed 3 days after her young were weaned. The glands showed much irregularity. Evidently several of the glands stopped functioning considerably sooner. The 2nd gland was the one least regressed. This gland was similar in every respect to that showing the early regressive changes described before. A small abnormal area was observed on the 5th gland. It consisted of a very small lobule in which the alveoli did not show any regression but contained numerous mitotic figures. Although the epithelial cells did not show secretory activity the lumens contained dense, deeply staining secretion. The area was not malignant but was definitely different from the areas which can be considered "normal" at this stage. The glands of 3 other dilute brown animals were examined in a resting state. One of them (No. 3214) showed an area of acute mastitis in the 5th gland. All of them showed a few small groups of alveoli which persisted through the regressing period.

The glands of 3 C57 black animals were examined and showed uniform normal regression. All the animals examined during this period were between the ages of 6 and 8 months.

Third Pregnancy: Dilute brown No. 6481 which was pregnant for 7 days showed the usual increased mitotic activity and rapid development of alveoli. There were a few small lobules which in contrast with this consisted of fully developed alveoli, the epithelial cells of which showed functional activity. One of these

glands consisted of well developed, functioning cells. On the 4th gland a microscopic area was observed where the epithelial cells were not functioning but rapidly dividing. In some places the lumen was being obliterated by an overgrowth of epithelial cells. While in most places the basement membrane was intact, in some places it had been broken through and the epithelial cells were seen in the process of invading the surrounding tissues and showed definite signs of carcinoma. The stroma was fibrous (Figs. 15 and 16).

One black animal examined at the corresponding stage showed acute mastitis in 1 gland. Two others proved to be normal. The animals included in this group were between the ages of 8 and 13 months.

Fourth Lactation: Dilute brown No. 3951 had no palpable tumor, but suspicious areas were noticed under the dissecting microscope on the left 1st and right 3rd glands. Sections showed that all the glands were lactating. The suspicious area on the left 1st gland was a small fibroadenoma. The stroma was very dense and at one place near the periphery showed lymphocytic infiltration. The small nodule on the right 3rd gland was an adenocarcinoma with fibrous stroma. The tumor was not encapsulated and was in the process of expansion by peripheral growth (Fig. 17).

All the glands of 2 other dilute brown and 2 C57 black animals examined at this stage were normal lactating glands. These mice were between the ages of 8 and 9½ months.

Resting after Fourth Lactation: Dilute brown No. 2585 showed acute mastitis with diffuse polymorphonuclear leukocytic infiltration on the 5th gland. In all other glands the regression was normal and uniform.

On the 4th gland of dilute brown No. 2796 a small area was considered suspicious under the dissecting microscope. After sectioning it was found to be composed of atypically arranged ducts and alveoli of greatly varied size. Many of these were composed of solid cords of cells and several of them had broken through the normal boundaries and were definitely malignant. Mitotic figures were numerous. The stroma showed slight fibrosis and lymphocytic infiltration. The area was not encapsulated (Fig. 18). On the 3rd gland a small area showed signs of chronic inflammation. In some of the alveoli in this area the epithelial cells changed into

functioning normal glands. Three others showed 1 or more glands in the process of regression. In these regressing glands the ducts proximal to the nipple were distended, the amount of adipose tissue was increased and the alveolar epithelium showed irregularities. In 1 mouse (No. 14403) a small area of acute mastitis was noted. All these animals were between the ages of 8 and 11½ months.

Resting after Third Lactation: All the glands of the dilute brown and black animals examined in this group completed their regression and were in a resting stage. In all of them the adipose tissue was greatly increased and the epithelial elements were at their minimum. Three dilute brown animals showed a few scattered groups of alveoli in several of their glands where the secretory activity persisted. Such areas were surrounded by normal adipose cells.

Dilute brown No. 4353 had a tumor on the 3rd gland which although small was large enough to be palpable. Microscopic examination showed an adenocarcinoma with hemorrhagic cysts and some necrosis. The adenoid structure consisted of small alveoli and had some resemblance to the glandular structure of a gland at the first half of pregnancy. The glands of this animal also contained several small areas where groups of alveoli persisted without completing their regression.

Two C57 black mice showed normal resting gland structure. All mice in this group were between the ages of 7 and 8 months.

Fourth Pregnancy: Dilute brown No. 4127 had been pregnant for 7 days when killed. A microscopic abnormal area was found on the 2nd gland. It consisted of an increased amount of epithelial cells changing and enlarging the normal outline of a few small ducts and alveoli. The area was surrounded by fibrocytes. Mitotic figures were numerous (Fig. 14).

Dilute brown No. 8095 had been pregnant for 10 days. A small palpable tumor was noticed on the 1st gland. Microscopic sections showed a rapidly growing adenocarcinoma with many mitotic figures.

Adjacent to this nodule there were some alveoli that contained secretion. The presence of a few small lobules with persistent secretory activity was noted on the 3rd and 4th glands.

Dilute brown No. 4165 was pregnant for 19 days and all the

TABLE I
Dilute Brown

Number of animal	Age in months	Abnormalities	Number of animal	Age in months	Abnormalities
<i>First Pregnancy</i>			<i>Third Pregnancy</i>		
2664	2		6481	7	Metaplasia, chronic and acute mastitis
2819	-3		3041	7	
2483	-4		3283	7	
2808	3		10807	9½	Abscess, chronic mastitis and metaplasia
2112	3				
2662	-3				
2502	2+				
<i>First Lactation</i>			<i>Third Lactation</i>		
78335	3		92675	-11	Chronic mastitis
2878	3+	Small areas of acute mastitis	2816	11½	
78261	4		97374	6	Infestation with mites
2887	3+				
2883	3+	Small areas of acute mastitis			
2916	3	Small areas of acute mastitis			
<i>Regression after First Lactation</i>			<i>Resting after Third Lactation</i>		
2815	4	Small areas of acute mastitis	2826	8	Small areas of persistent secretory activity
4187	4		2818	8	Same as above
4108	4		10806	8	Same as above
5535	-5		4353	8	Adenocarcinoma
97371	5½				
<i>Second Pregnancy</i>			<i>Fourth Pregnancy</i>		
4540	5	Adenocarcinoma	4127	8	Thickened wall of ducts
5430	5	Persistent growing area	8095	-13	Adenocarcinoma, persistent secretory activity
1688	5		4165	-9	Adenocarcinoma
<i>Second Lactation</i>			<i>Fourth Lactation</i>		
96624	-6		3951	9	Fibroadenoma
5636	6	Chronic mastitis	3882	9	Adenocarcinoma
3310	4		4006	8	
3320	4				
<i>Regression and Resting after Second Lactation</i>			<i>Resting after Fourth Lactation</i>		
5432	8	Persistent growing area	2585	9	Acute mastitis
3425	6		2796	9	Adenocarcinoma, chronic mastitis and metaplasia
3214	-7	Acute mastitis			
3003	7				
			<i>Fifth Pregnancy</i>		
			7110	13	Carcinoma simplex, adenocarcinoma
			7856	12	Thickened wall of ducts and alveoli
			8061	12	Carcinoma simplex, persistent secretory activity
			7869	13	Fibroadenoma

cornified stratified squamous cells, due to metaplasia. All of the glands contained some small persistently secreting groups of alveoli.

The glands of 2 black mice were examined at this stage. All showed normal structures. These animals were between the ages of 9 and 12 months.

Fifth Pregnancy: Dilute brown No. 7110 was pregnant for 8 days. Microscopically the sections showed several small areas where alveoli containing quite dense secretion persisted, evidently from the previous lactation period. The main ducts in the 1st and 5th glands were distended by retained secretion. In the 2nd gland a very small, definitely malignant area was observed. This area showed some rapidly and densely growing epithelial cells without alveolar arrangement, developing around a small papilliferous cystic adenocarcinoma. The stroma was fibrous but the area was not encapsulated. None of the elements in the malignant area contained retained secretion. A somewhat larger malignant area was present in the 5th gland. This area was noticed under the dissecting microscope but was not large enough to be palpable. Here the epithelial elements were arranged in adenoid form. Some alveoli contained retained secretion; others contained no secretion and were growing very rapidly. In a few of them near the periphery calcium deposit was found. Several small cysts contained necrotic material. The stroma was quite fibrous; the tumor was not encapsulated.

Dilute brown No. 7856 was pregnant for 9 days. In the 5th gland a small group of ducts and developing alveoli were growing atypically. The epithelial cells were rapidly multiplying and were lining the lumens with several rows of cells. The basement membrane was still intact. The surrounding stroma showed increased fibrosis. The rest of the glands did not show any abnormalities.

Dilute brown No. 8061 was pregnant for 15 days. The development of all the glands was quite uniform. A few ducts contained some retained secretion. In the 4th gland a small abnormal nodule was noticed microscopically. It was a carcinoma simplex and consisted of a disorganized growth of large atypical epithelial cells. The nodule was surrounded by a fibrous capsule which showed some lymphocytic infiltration (Fig. 19).

Dilute brown No. 7869 was pregnant for 19 days. All the glands

Comparing the glands of these dilute browns with the glands of 3 blacks which were included in this group, the unevenness in the development of the dilute brown mice is becoming more and more evident. All these mice were between the ages of 11 and 13 months.

Murray⁸ made a detailed analysis of the breeding behavior of the dilute brown strain. According to his data the frequency distribution curve representing the age of mice at the appearance of tumor reaches its mode at 10½ months. As the animals described in the last group had already passed through this age the investigation has not been carried further.

The age of the animals and the abnormalities observed in them from the period of the first to the fifth pregnancy are presented in Tables I and II.

DISCUSSION

From the data presented above it is evident that comparing the mammary glands of the dilute brown high tumor strain with the C57 black low tumor strain, structural differences can be detected before the appearance of palpable tumors. These differences manifest themselves in two main ways:

1. During the latter part of pregnancy a uniform change takes place in the epithelial cells of the glands of the black animals — cell multiplication ceases and secretion starts.

In the dilute brown mice this change is not always uniform and small groups of epithelial cells sometimes persist in cell multiplication.

2. During the period of regression the epithelial cells of the glands of the black animals gradually cease to secrete and the alveoli regress completely.

In the dilute brown mice these changes are not always uniform — small groups of alveoli often persist and sometimes some of the epithelial cells keep on secreting.

As mentioned previously, enough experimental data have accumulated to prove that although breast development is brought about during pregnancy by estrin and luteal hormones, the secretion of milk can be effected only through the agency of an anterior pituitary hormone — prolactin. It seems important that a proper balance should be maintained between the hormones that stimulate

were composed mostly of secreting epithelial cells. A small fibroadenoma was found on the 5th gland. The epithelial cells filled up the lumens and enlarged the glandular structure. The basement membrane was not broken through. Mitotic figures were numerous. The stroma showed increased fibrosis. The tumor was not encapsulated (Fig. 20).

TABLE II
C57 Black

Number of animal	Age in months	Abnormalities	Number of animal	Age in months	Abnormalities
First Pregnancy			Regression and Resting after Second Lactation		
14135	-2		14040	8	
14151	2+		14248	7	
13921	2+		14313	7	
14277	2+		Third Pregnancy		
14242	2+		15643	8	
14312	2+		16218	8	
First Lactation			283	8	
Third Lactation			Third Lactation		
14328	2+	Acute mastitis	14403	8	Acute mastitis
14327	2+		16051	8	
13594	4+		16340	7	
13695	4+	1192	10		
13920	3+	Acute mastitis	Resting after Third Lactation		
11907	4		14136	8	
Regression after First Lactation			14153	7+	
Fourth Pregnancy			Fourth Pregnancy		
15602	4	Acute mastitis	18609	13	Acute mastitis
15334	4		14702	10	
15628	4		19318	11	
21484	3		Fourth Lactation		
Second Pregnancy			14440	9	
Fourth Lactation			2768	9½	
146	5		Resting after Fourth Lactation		
147	5		15357	12	
280	5+		14202	10	
281	5		Fifth Pregnancy		
282	4+		19312	12	
Second Lactation			19313	-12	
Fifth Pregnancy			19838	11	
15664	4	Obstruction of 1 nipple			
15983	4				
15888	-5				
10526	-5				

brown mice. Cornified cells filled the lumens and the surrounding area showed fibrosis and signs of chronic mastitis.

Small areas of acute mastitis were observed most often during lactation and were found in the glands of the black animals as well as in the dilute brown animals.

SUMMARY AND CONCLUSIONS

A comparative morphological study of the mammary glands of the Little-Murray dilute brown high tumor strain and the C57 black low tumor strain of mice showed that the glands of the high tumor strain do not respond so uniformly to the endocrinal influences that regulate the progressive, functioning and regressive changes of the gland as do those of the low tumor strain.

In the high tumor strain groups of cells may persist in cell division, while all the others are already functioning; or fail to regress, sometimes keeping on functioning while all the others have undergone regression. The persistent mitotic activity of groups of cells leads to early malignant changes.

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cell division and function. This balance might be upset in several ways. (a) One or the other hormone might be produced in excess quantity. In this case the effect of the resulting disturbance would probably be more widespread. (b) Some groups of cells might be able to store up or unable to eliminate certain hormones. This might render them incapable of utilizing the effect of other hormones.

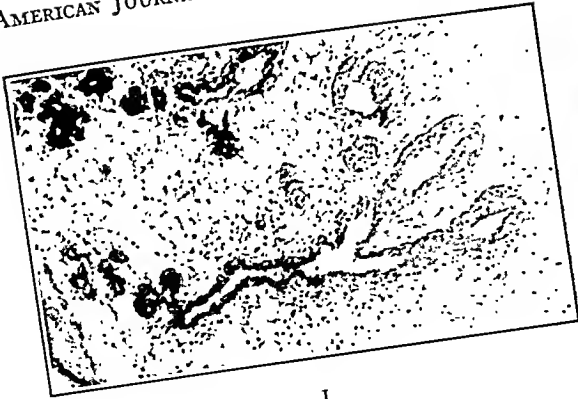
In a recent paper Geschickter and Lewis⁹ present an interesting study of pregnancy and lactation changes in fibroadenoma of the breast in humans and state: "The failure of the fibroadenomatous tissue to respond to the same extent and in the same manner as normal breast tissue to all phases of pregnancy and lactation suggests that the tumor is more sensitive to certain hormones than to others." In the present study this "failure to respond" was found to be present in certain groups of cells even before the cells were atypical or produced excessive growth.

The failure of small groups of epithelial cells to respond to the stimulation of prolactin and persist in cell multiplication during the latter part of pregnancy seems to lead to carcinoma in the dilute brown animals.

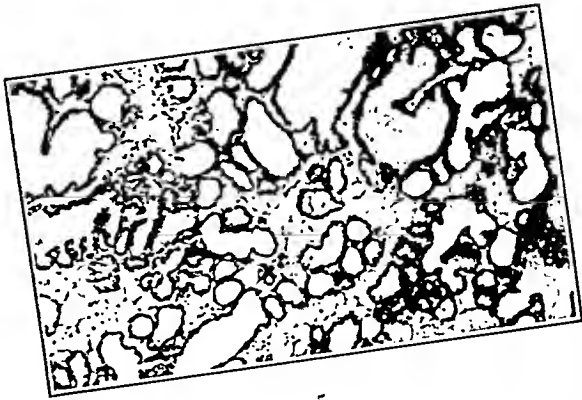
Structurally these persistently growing areas are composed at an early stage of small epithelial cells with a relatively large nucleus and a small amount of cytoplasm surrounding a small lumen. Mitotic figures are numerous. The surrounding stroma shows increased fibrosis. Later the epithelial cells fill up the lumen, enlarge the whole outline of the duct and finally break through the basement membrane. Although the fibroblasts which surround these areas at the early stages also show proliferation, they soon lag behind in development and the epithelial cells often penetrate into the surrounding adipose tissue. Cyst formation if present at all in this type of tumor is usually due to necrosis or rupture of blood vessels.

The failure to complete regression and the persistence of secretory activity of small groups of epithelial cells do not seem to be so dangerous from the point of view of malignant changes. In such areas mitotic figures are infrequent. If malignant changes of these areas do take place they probably develop slowly and later in the life of the animal.

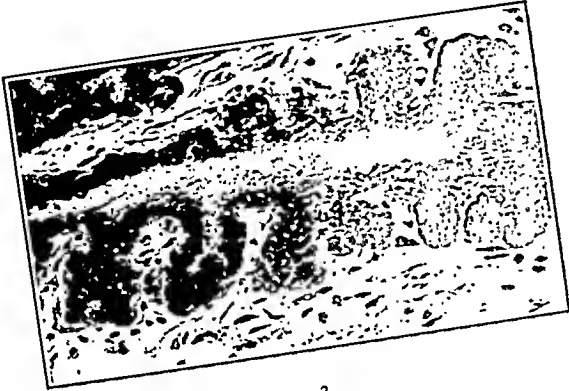
Metaplasia was noted in several of the glands of the dilute



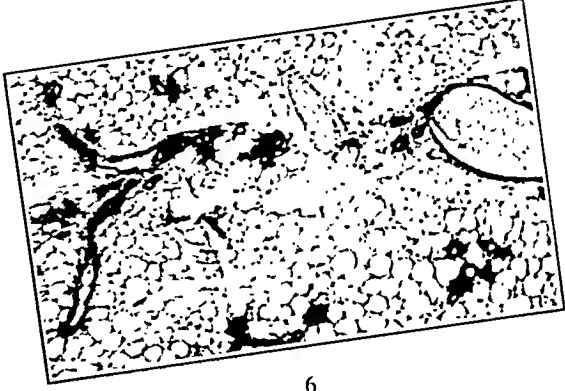
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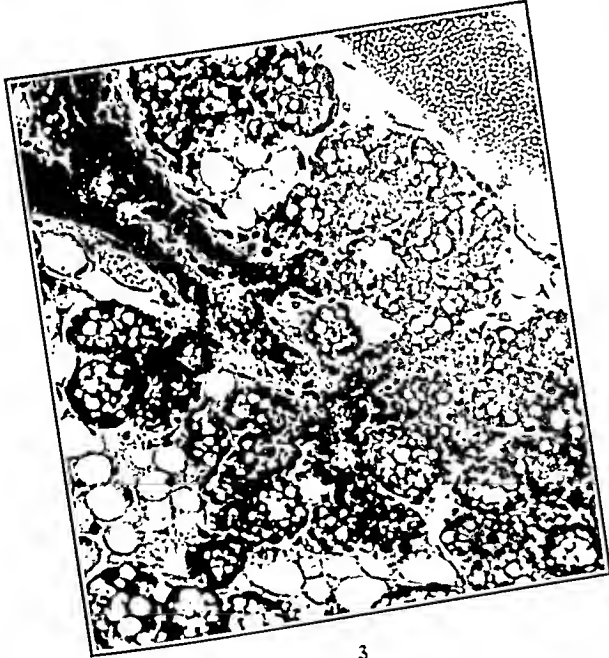
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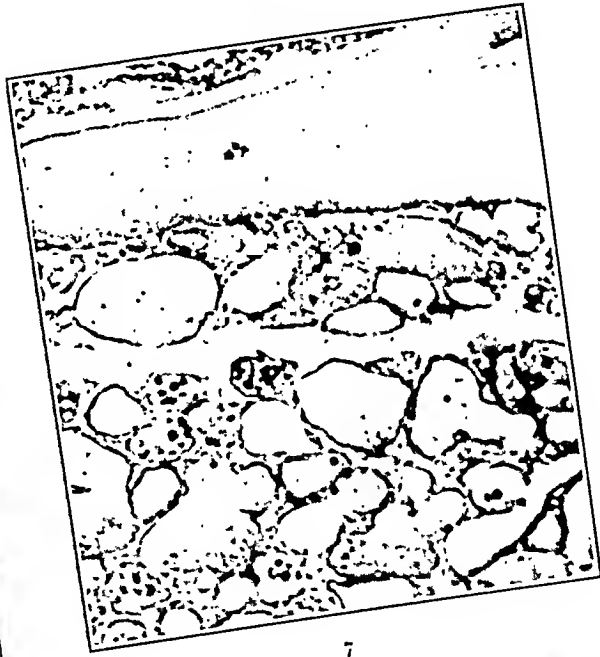
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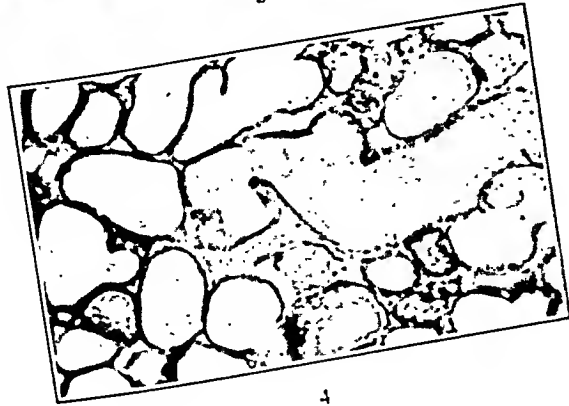
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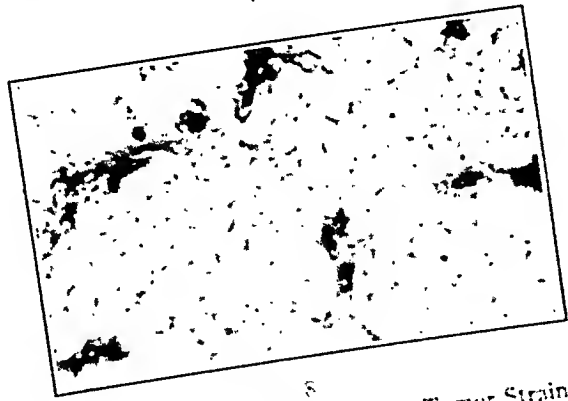
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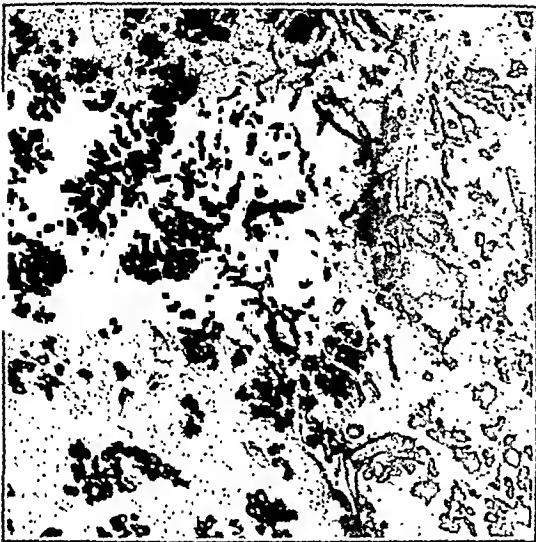
Mammary Gland in High and Low Tumor Strain

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DESCRIPTION OF PLATES

PLATE 135

- FIG. 1. End bulbs on the mammary gland of an 8 weeks old dilute brown mouse. $\times 65$.
- FIG. 2. Rapidly developing mammary gland on the 11th day of pregnancy. $\times 200$.
- FIG. 3. Mammary gland showing secretory activity on the 20th day of pregnancy. Note the decrease of adipose cells and the increase of the glandular elements. $\times 200$.
- FIG. 4. Mammary gland of a mouse lactating for 7 days. Note the almost complete absence of adipose cells. $\times 100$.
- FIGS. 5 and 6. Glands of the same C57 black mouse that was lactating for 20 days. Figure 5 is the 4th gland and shows secretory activity. Figure 6 is the 3rd gland and shows regression of the glandular elements and an increase of adipose tissue. $\times 50$.
- FIG. 7. Mammary gland 24 hours after lactation had stopped. Note the round bodies in the ducts and alveoli. $\times 100$.
- FIG. 8. Mammary gland at resting stage. Adipose cells greatly increased—the glandular elements reduced. $\times 100$.



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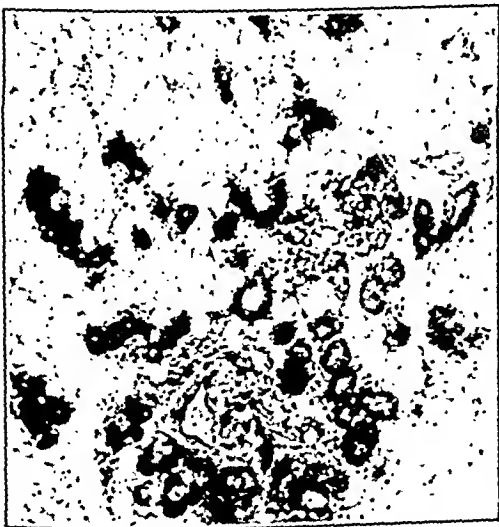
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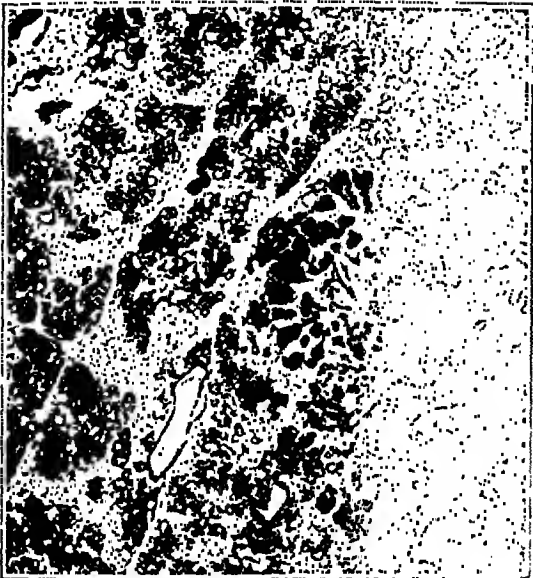
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PLATE 136

- FIG. 9. Mammary gland of dilute brown No. 4540. This animal was pregnant for 15 days and secretory activity has started in the cells of the normal area. The abnormal areas are deeply staining and do not show secretory activity. $\times 25$.
- FIG. 10. Part of Figure 9 under higher magnification. In the lower left corner a normal lobule is seen. The upper right corner shows malignant changes in the abnormal area. $\times 250$.
- FIG. 11. Mammary gland of dilute brown No. 5430. The animal was pregnant for 19 days and secretory activity was well established. In the abnormal area the cells are not functioning but are still actively dividing. $\times 25$.
- FIG. 12. Part of Figure 11 under higher magnification showing normal part at the lower left and abnormal part above it. $\times 75$.
- FIG. 13. The gland of dilute brown No. 6481 at 7th day of pregnancy. The abnormal area shows a few alveoli with persistent, functional activity and metaplasia of the glandular epithelium to cornified, stratified squamous epithelium. $\times 100$.
- FIG. 14. Dilute brown No. 4127 pregnant for 7 days showing the thickened wall of a few ducts and alveoli. $\times 75$.



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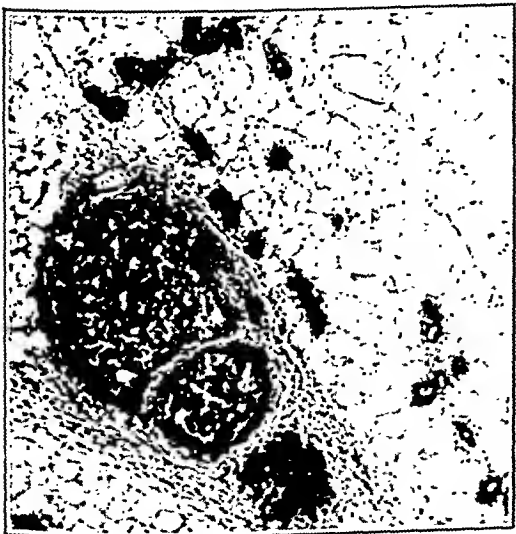
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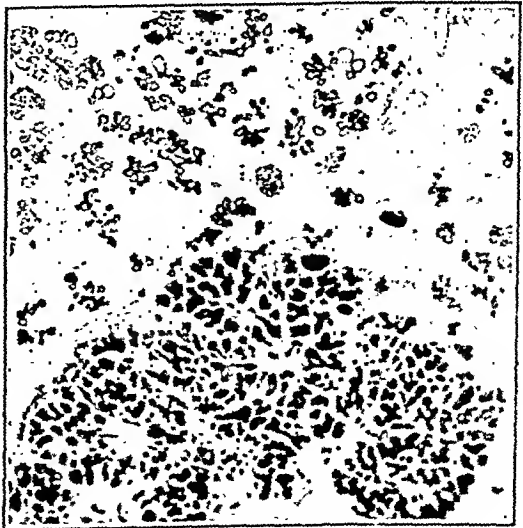
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PLATE 137

FIG. 15. Dilute brown No. 4165 pregnant for 19 days showing an abnormal growing area surrounded by a normal functional area. $\times 25$.

FIG. 16. Part of Figure 15 under higher magnification. The normal area is on the left; the abnormal on the right side of the picture.

FIG. 17. The gland of dilute brown No. 3951 lactating 1 day shows an adenocarcinoma with fibrous stroma. $\times 75$.

FIG. 18. The gland of dilute brown No. 2796 at resting stage, showing a small adenocarcinoma. $\times 25$.

FIG. 19. The gland of dilute brown No. 8061 pregnant for 15 days and showing a small carcinoma simplex which is encapsulated. $\times 100$.

FIG. 20. The gland of dilute brown No. 7869 pregnant for 19 days and showing a fibroadenoma. $\times 25$.

STUDIES ON THE INFECTIOUS AGENT OF INCLUSION BLENNORRHEA *

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INTRODUCTION

The acute conjunctivitides occurring in the newborn were originally designated indiscriminately as "ophthalmia neonatorum," and until the advent of bacteriological methods there was little opportunity of demonstrating their occurrence in various forms. With the discovery of the gonococcus by Neisser ¹ in both the urethral discharge of adults and the conjunctival exudate of infants, the most severe and common variety became classifiable and identified as blennorrhea. In rapid succession the different organisms etiologically related to the different types of ophthalmia neonatorum became known, thus permitting a logical and feasible classification of this pleomorphic clinical condition. In spite of the advances made, however, there were repeatedly cases of blennorrhea which defied bacteriological analysis since no visible or cultivable organisms were present, and although this particular manifestation had been commented on by several observers,^{2,3,4} it was Morax ⁵ who differentiated it by name when he termed it "conjunctivite amicrobienne."

With the discovery later by Halberstaedter and Prowazek ⁶ of the cytoplasmic inclusion of the epithelial cell in trachoma it was a natural sequence to search for and, as it happened, to discover ^{7,8} morphologically identical structures in the "amicrobienne" instances of ophthalmia neonatorum. Without delving too deeply into the historical background it is sufficient to say only that this clinical condition was soon established as an entity and henceforth it became identified as inclusion blennorrhea,⁹ with the concept predominant in the minds of most workers that the epithelial inclusion was the causative agent.[†]

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† For a detailed account of the clinical and general aspects of inclusion blennorrhea the reader is referred to a later communication, now in press in the *American Journal of Ophthalmology*.

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stances, immediately on return to the laboratory. In the majority of instances the delay from clinic to laboratory was very short, since both departments are in the same building. The ground material was used for a variety of purposes, modified to a further extent in certain cases, as will be described below. In testing infectivity of the tissues, inoculations were made by direct swabbing from patient to animal, by swabbing with the suspension of tissues, or by injection, which was accomplished by multiple pricking of the conjunctiva with a charged needle and allowing material from the syringe to drip over the scarified surface and then introducing about 0.2 cc. of the suspension subconjunctivally.

EXPERIMENTAL

In initiating this study it was necessary to determine the transmissibility of inclusion blennorrhea to animals. While former workers had already established its infectivity in apes and monkeys,¹¹⁻²² the number of animals inoculated was small and the descriptions of the experimental disease lacked detail. Consequently it was decided to restudy the problem of transmissibility. In preliminary experiments tissues from infants were inoculated into rabbits, guinea pigs and rats, both conjunctivally and by different routes, without inducing any reactions perceptible by clinical observation. Attempts to adapt the infectious agent in white mice, Swiss mice and Bar Harbor mice (C-57) by successive intraperitoneal or intracerebral passage were complete failures, and examination of various organs, particularly the spleen and the brain, showed no gross or histological changes. Inclusions were sought for but were never detected in the tissues of these animals.

Transmissibility to Apes and Monkeys

In transferring material from the infant to monkeys or apes, as described above, it was possible to induce experimental infection of the conjunctiva. Of a total of 50 monkeys (*Macacus rhesus*) inoculated with unaltered human tissues, 25 were infected successfully. The variations in the transmission of the disease are in part due to the tissues themselves, and in part to the variation in individual susceptibility of monkeys.* For a few days following

* A more complete report of the factors influencing the infectivity of a given tissue will be published later in the *American Journal of Ophthalmology*.

Contiguous studies undertaken in this laboratory on the etiology of trachoma¹⁰ indicated that inclusion blennorrhea and trachoma possess certain analogies, as others, in fact, have pointed out in the past. As a consequence of the study of these two diseases it was felt that an effort should be made to prove their separate identity, if possible. After five years of intermittent study it is felt that sufficient information has been acquired to justify a report on the nature and etiology of inclusion blennorrhea.

METHODS

Patients: The patients under observation in this study were for the most part attending the ophthalmological clinic of Washington University *; 3 came from the St. Louis Maternity Hospital, and 1 from the Bethesda Hospital of St. Louis. This will convey in a measure the infrequency of this disease, when it is considered that careful search during this interval in these institutions yielded only 22 patients. Diagnosis was made tentatively on clinical grounds but it was always verified later by a search for the characteristic inclusions, and only those patients with relatively numerous lesions were utilized for experimental study.

Material: Material for study consisted of a suspension of tissue fragments obtained by scraping the conjunctiva of the everted lid. This was accomplished by first removing the exudate with soft cotton gauze, sometimes supplemented by short saline irrigation. The eye was anesthetized with holocaine or pontocaine and the conjunctiva of the everted lid was scraped with a sterilized platinum spatula of the Lindner type. The tissues thus removed were emulsified in 1 cc. of veal infusion broth (pH 7.6 to 7.8). Since the acute stage of this disease lasts 2 weeks or more, it was possible to return to the same patient a number of times at intervals of a few days or a week, thus obtaining from some of them a relatively large quantity of infectious material. It should be pointed out that the scraping technique was used for therapeutic purposes, so that the procedure was not a condition imposed by this study.

Inoculations: The suspension of tissues was always triturated under sterile conditions, without the intervention of abrasive sub-

* The writers gratefully acknowledge the cooperation and assistance of Dr. L. T. Post of this university in procuring patients and extending the facilities of his clinic toward the completion of this work.

mals recovering from experimental inclusion blennorrhoea were inoculated at a later date with active material from trachoma. It was not possible to show that the former infection offered any protection to the later inoculation. This is in contradistinction to the results published by Lindner²³ but later unconfirmed by Thygeson and Mengert.²¹

The histological changes stimulated by the experimental process were essentially those of lymphoid hyperplasia and as such possessed not specific but merely the general characteristics accompanying follicular hypertrophy. In any event, examination of sections revealed follicles varying in size, composed for the most part of small lymphocytes and plasma cells occasionally surrounded by a thin collar of fibroblasts. In the earlier stages the conjunctival epithelium was frequently undergoing desquamation and many individual cells showed a marked degree of swelling or ballooning. As the infection progressed into the chronic phase the epithelium was regenerated and in certain areas thrown into invaginations due presumably to the pressure exerted by the larger sized follicles. Scarring was never observed so that histologically as well as clinically experimental inclusion blennorrhoea must be regarded as just as benign a disease as its spontaneous counterpart in the infant.

As already stated above, inclusion blennorrhoea is identified pre-eminently by the cytoplasmic inclusion of the epithelial cell. Consequently, repeated search was made for its presence in the infected monkey. In the limited sections prepared for histological examination inclusion bodies were not found. In scrape smears regularly made of the conjunctiva inclusion bodies were found in only 2 monkeys. The inclusions were not numerous and they were located with difficulty. In 1 animal inclusions were found on the 5th and 8th days following inoculation, and in the 2nd animal only on the 4th day. Other observers,^{9-18,20-22} have found them with relative ease, particularly in baboons, so that Thygeson²⁰ suggests that *M. rhesus* is a poor animal for the observation of inclusions. Whatever the explanation it must be admitted that there are a number of instances in which inclusions fail to appear in the experimental transmission of a disease to a foreign host.

inoculation there was a varying but never a marked degree of mucopurulent discharge, mild hyperemia, slight congestion and occasional swelling of the lids. These were not regarded as prodromal symptoms since they were observed frequently even in animals not infected. It was considered more probable that the reaction was in response to the bacteria usually accompanying inclusion blennorrhea, and because of their non-pathogenic nature they were easily eliminated.

Successful infection was determined by the appearance of follicles, which were detected as single or small clusters of translucent, discrete and circular elevations, at first in the retrotarsal folds. This sign was accepted as the first evidence of specific infection, so that on this basis the period of incubation was found to vary in different animals from 5 to 13 days. Slightly more than half the animals, however, became infected within a week after inoculation with the human tissues. The follicles gradually increased in number and were characterized by their uneven distribution, variable size and occasional vascularization, so that some follicles had a distinctly reddish cast. The infection soon became a chronic process, with the general appearance unchanged until healing set in, when the follicles commenced to recede gradually. While the duration of the experimental infection varied considerably from a minimum of 2 weeks to a maximum of 7 months, more than half the animals recovered within 6 weeks.

At no time during the experimental disease did the infection extend to the tarsus, bulbar conjunctiva or cornea. The reaction was always follicular, never papillary, as commonly observed in infants, and irregularly there appeared to be a greater degree of folliculosis in the lower lids. On recovery the conjunctiva assumed its originally normal appearance and an exacerbation of clinical symptoms was never seen.

In 1 of 2 Sphinx baboons infected the reaction was similar to that in the monkeys and there was an unmistakably greater involvement of the lower lids.

A study was made of serial passage of the experimental disease, but it was found that the infectious agent was lost in two to three transfers from animal to animal. Following recovery it was determined that such animals acquire no increased resistance to reinfection. A corollary experiment was conducted in which ani-

etiology with filtrates of active tissues, and to extend the experiments of former investigators. Tissues for this purpose were obtained by scraping the everted conjunctiva and emulsifying in broth the tissues thus obtained. After grinding the suspensions were centrifugated for 3 to 5 minutes at 1500 r.p.m. to remove heavier particles. The supernatant fluid was filtered at a pressure of 20 cm. Hg. The filtrates were seeded on blood agar to determine sterility and the filters were tested with young broth cultures of *B. prodigiosus*. The filters consisted principally of collodion membranes with average pore diameters varying from 0.6μ to 0.69μ , and in two experiments Berkefeld V candles were employed.

In this way ten experiments were performed, but in four the original material proved to be inactive for monkeys. The data bearing on these experiments are summarized in Table I. It will be seen that in two of the six successful experiments (B-11b and B-17), tissues originally infectious no longer retained their activity when filtered through collodion membranes measuring 0.65μ A.P.D. In the remaining trials the infectious agent traversed the membranes (0.63μ to 0.65μ) in two experiments (B-15 and B-16). In the last two attempts the infectious agent was recovered by Berkefeld filtration in one experiment (B-18) and by both Berkefeld and collodion filtration in the other (B-19a and b). In one of the later filtrations the tissues studied were pooled from 5 infected monkeys. It is obvious then that when the proper conditions are met the incitant of inclusion blennorrhoea is filterable.

On further analysis it was found that there is, however, a distinct loss in degree of activity during filtration. Thus, of 18 monkeys inoculated with unfiltered material 15 were infected, while of 21 inoculated with corresponding filtrates only 6 were infected. It may well be, therefore, that the differences in infectivity observed in filtrates from different infectious tissues are due to a partial retention of virus particles which in one instance may be sufficient to carry the concentration of infectious agent beyond its range of activity, while in another instance a similar degree of retention may have no effect on ultimate infectivity because more virus is present originally.

Bacteria Cultivable from Inclusion Blennorrhoea

Having verified the transmissibility of inclusion blennorrhoea to monkeys it was proposed to determine, if possible, the nature of the agent responsible for the evolution of the infection. The first step in this direction was taken by means of bacterial cultures from infected infants. Ten patients were studied with care, although routine cultures were carried out on all. Conjunctival scrapings emulsified in veal infusion broth were inoculated on rabbit blood agar, horse serum agar, and Noguchi's leptospira semisolid agar. In a few cases ascitic fluid containing fresh rabbit kidney and sealed with sterile vaseline was also used. Incubation of seeded mediums was carried out at 30°C. and 37°C., and in most instances up to 5 days.

It was soon realized that the organisms cultivated, regardless of the medium employed, were those usually associated with the flora of the normal conjunctiva. Thus, staphylococci were isolated in greatest frequency and numbers, with diphtheroids following closely. Moreover, no variations in flora were noted that might be correlated with the variations in infectivity of tissues. The important fact is, however, that none of the organisms cultivated was capable of inducing in monkeys reactions resembling experimental inclusion blennorrhoea. It was concluded, therefore, that the bacteria recovered from infected infants were adventitious and played no primary or causal part in the spontaneous disease.

Filterability of the Infectious Agent

A study of the literature reveals that the incitant of inclusion blennorrhoea was found to be filterable in a total of six experiments, twice through Berkefeld filters^{17, 24} and four times through colodion membranes. Thygeson,²⁰ who was first to employ the latter, showed that on two occasions the agent traversed membranes measuring 0.7 μ A.P.D., and Tilden and Gifford²² confirmed the observation with membranes measuring in one experiment 0.62 μ , and in another, 0.46 μ A.P.D. On the other hand, Lindner²³ found that in four experiments Berkefeld filters retained the infectious agent.

With the tentative elimination of bacteria in the evolution of inclusion blennorrhoea it was proposed to continue the study of the

Cultivability in Tissue Cultures

Since it was shown that the infectious agent of inclusion blennorrhea is able to penetrate both Kieselguhr and collodion filters, and that the agent present in such filtrates is incapable of cultivation in bacteriological mediums, it became necessary to attempt propagation in tissue culture. The methods employed for this purpose were the same as those described in a former communication on trachoma.²⁵ Scraped tissues from patients were washed through several changes of Tyrode's solution to eliminate the contaminating bacteria usually present. While not completely effective, it was possible in this way to obtain a sufficient number of bacteriologically sterile tissues to permit their uncontaminated cultivation. In some experiments filtered material was also used. Inoculations were made from 7 different patients, but material for tissue cultivation was always taken more than once, and in some cases more than four times, from individual infants, thus increasing manifold the actual number of cultures. The cultural methods consisted of minced chick embryo, minced rabbit testicle or kidney, plasma clot with normal human or normal rabbit plasma seeded with conjunctival cells from the patient or with normal rabbit tissue impregnated with filtrates of infected human tissues. Cultivation was similarly attempted in the fertile hen egg. Tissue cultures of different ages were subsequently inoculated in monkeys, either singly or pooled, and a microscopic study was made to determine the presence of abnormal cellular changes as well as structures indicative of virus activity.

A summary of the data on these experiments indicates that neither inclusions nor their constituent elementary or initial bodies were ever observed in smears or sections of the tissue cultures. None of the cultures, moreover, was capable of inducing infection in the monkeys inoculated, despite the fact that some of the animals selected were subsequently demonstrated to be susceptible to experimental inclusion blennorrhea. It appears, therefore, that the infectious agent of inclusion blennorrhea is incapable of multiplication under the conditions of tissue cultivation described above.

TABLE I
Infectivity of Filtrates of Tissues from Inclusion Blennorrhoea

Experiment	Source of material	Method of filtration	Culture of filtrate	Infectivity of tissues	
				Unfiltered	Filtered
B-11(b)	Infant	Elford membrane (A.P.D.-0.65 μ)	No growth	Infected 2 of 2 monkeys *	Inactive in 2 monkeys
B-17	Infant	Elford membrane (A.P.D.-0.65 μ)	No growth	Infected 3 of 3 monkeys	Inactive in 3 monkeys
B-15	Infant	Elford membrane (A.P.D.-0.63 μ)	No growth	Infected 2 of 4 monkeys	Infected 1 of 4 monkeys
B-16	Infant	Elford membrane (A.P.D.-0.65 μ)	No growth	Infected 4 of 4 monkeys	Infected 2 of 4 monkeys
B-18	Infant	Berkfeld V	No growth	Infected 2 of 2 monkeys	Infected 1 of 2 monkeys
B-19a	Pooled from 5 monkeys	Elford membrane (A.P.D.-0.65 μ)	No growth	Infected 2 of 3 monkeys	Infected 1 of 3 monkeys
B-19b		Berkfeld V	No growth		Infected 1 of 3 monkeys

* The species of monkey used was *M. rhesus*.

TABLE II
Contrasts and Similarities between Trachoma and Inclusion Blennorrhoea

	Incubation in days	Duration	Character of onset	Effect of infection on			Inclusions	Effect of silver nitrate	Recurrence	Occurrence in other tissues
				Lids	Cornea	Vision				
Natural infection in man: Trachoma	6 to 30	Permanent	Insidious	Follicular or papillary reaction with scarring	Infiltration, pannus, ulceration, scarring	Impairment	Identical cytoplasmic inclusions of epithelial cell; considerably more common in inclusion blennorrhoea	Curative	Common	Eye only
Inclusion blennorrhoea	3 to 14	Few months to a year	Acute	Same but without scarring	None	None		None	Rare	Genital tract as well as eye
Experimental infection in monkeys: Trachoma	5 to 30	Several months to 2 or 3 years	Gradual	Follicular only	None	None	None *	Not known	Never observed	
Inclusion blennorrhoea	5 to 13	Few weeks to few months	Gradual	Follicular only	None	None	Rare	Not known	Never observed	

* Inclusion bodies have never been found in this laboratory in preparations from monkeys infected with trachoma, but other authors report their occurrence under such conditions. In experimental inclusion blennorrhoea, inclusions have been found here as well as in other laboratories, and while rare even in this infection, their frequency appears greater than in experimental trachoma.

DISCUSSION

The studies undertaken in the present investigation suggest that the infectious agent of inclusion blennorrhea is not bacterial. Indeed, it appears to be a virus capable of inducing specific infection in monkeys after passage through Berkefeld V and collodion filters (A.P.D. 0.63μ to 0.65μ), in this respect corroborating and amplifying experiments of certain former observers.^{17, 20, 21, 22, 24} The agent, however, is unable to grow in a variety of tissue cultural mediums, which may indicate in another way its viral nature.

The epithelial inclusions accompanying the disease are undifferentiable from those of trachoma, thereby suggesting to certain workers a relation even of identity between the infectious agents of the two entities. On the basis of inoculations in man, moreover, Wolfrum²⁶ concluded that the two conditions are actually manifestations of the same infection. His description of the experimental infection is so lacking in details as to prevent analysis of his reasons for the diagnosis. On the other hand, this was subsequently denied by Gebb²⁴ and later by Thygeson,^{20, 21} the latter demonstrating that inclusion blennorrhea appears as a conjunctivitis in adults recognized as swimming-bath conjunctivitis. Similarly, in an infection transmitted by accident to an attending nurse, it was observed in this laboratory²⁷ that the virus of inclusion blennorrhea does not induce trachoma in the adult, but, as Thygeson first reported, swimming-bath conjunctivitis. On rereading Gebb's experiments it is obvious that his results indicate a similar conclusion, the true diagnosis possibly escaping his attention, as well as that of others reporting accidental infection from infant to mother,²⁸⁻³⁰ because swimming-bath conjunctivitis was still too recently recognized an entity to receive universal acceptance. It seems at the present time, therefore, that inclusion blennorrhea represents the infantile response of the conjunctiva, and swimming-bath conjunctivitis the adult response to the same agent.

In order to bring out the contrasts and comparisons between the two diseases, a table (Table II) has been compiled to include the various characteristics of each. Analysis of the individual manifestations reveals that both in spontaneous and in experimental infection there is a shortening of incubation period and duration in inclusion blennorrhea. While in appearance the two diseases are

2. The infectious agent is incapable of serial transfer in monkeys and it stimulates no immunity in recovered animals.

3. Transmissibility to monkeys is not dependent on the bacteria present in and cultivable from active tissues of infectious patients.

4. The infectious agent, while losing a certain degree of activity, passes through Berkefeld V filters and collodion membranes with an A.P.D. of *ca.* 0.6μ .

5. It was not possible to propagate the infectious agent under several different conditions of tissue cultivation.

6. The indications are that the infectious agent of inclusion blennorrhoea is a virus.

7. The clinical evidence indicates that inclusion blennorrhoea is different from trachoma although their respective viruses may be similar or closely related biologically.

8. No evidence can be derived from this study either to affirm or refute the identity of the virus and the inclusion body of inclusion blennorrhoea.

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similar in the monkey, in man trachoma only induces cicatrization, corneal complications, impairment of visual acuity, and exhibits a marked tendency toward recurrence after apparent healing. The inclusions are found in greater numbers and in a greater proportion of patients with inclusion blennorrhoea. The same conclusion is tenable in experimental infection but on a more reduced scale. Silver nitrate has been found to be an effective therapeutic agent in trachoma but not in inclusion blennorrhoea. Trachoma, moreover, occurs only in the eye and its adnexa while inclusion blennorrhoea is essentially a genital infection transmitted from mother to infant *intra partum*.^{20, 21, 31, 32} While there can be no doubt, therefore, that the two ocular infections are clinically different it must be admitted that any and all of the differential characteristics may be due as much to variations in degree of pathogenicity of the strains of virus involved as to any actual distinctions in species. The inability of both viruses to survive serial animal passage, stimulate immunity and initiate artificial propagation, as well as the striking resemblance of the respective inclusions, suggest that the two viruses fall within the same or closely related biological groups.

Other authors have considered the infectious agent of inclusion blennorrhoea to be the inclusion body itself. The present study offers no information to support or refute this opinion. While the experiments of Thygeson^{20, 21} suggest that the elementary body is the virus and certain data on particle size of the virus (unpublished) obtained in this laboratory also intimate that this may be so, it appears to us that further study is needed before such a limited definition of the virus can be rendered conclusively. That the infectious agent is a virus is supported by the collective evidence of several investigators; to carry this statement further, however, takes the discussion outside the realm of inclusion blennorrhoea into the broader field of viruses in general, where the problem bearing on the identity of virus and inclusion is widespread and not particular to any special disease.

CONCLUSIONS

1. Inclusion blennorrhoea is transmissible to apes and monkeys as a self-limited follicular conjunctivitis without complications or sequelae.

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THE PHAGOCYTIC ACTIVITY OF HUMAN LEUKOCYTES WITH SPECIAL REFERENCE TO THEIR TYPE AND MATURITY *

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The polymorphonuclear neutrophiles are the most active phagocytes of the body. In an inflammatory exudate, regardless of the nature of the irritant, the polymorphonuclears are the first cells to arrive and to attempt its removal. The monocytes of the blood are also actively phagocytic. Schwarz¹ in an extensive review of the literature on the subject of eosinophiles found no agreement among the various investigators as to the phagocytic activity of these cells. Strumia and Boerner² in a recent report found the phagocytic activity of eosinophiles to be far less than that of the neutrophiles or monocytes, although a fair percentage of circulating eosinophiles showed phagocytosis. It is extremely difficult to recognize phagocytosis in basophiles because of the large basophilic granules within the cytoplasm of these cells. It is commonly believed that lymphocytes as such are not capable of phagocytosis. Strumia and Boerner² found no phagocytosis in lymphocytes. They studied a case of infectious mononucleosis and found no phagocytic activity in the leukocytoid leukocytes of this condition. The question whether or not lymphocytes ever possess phagocytic properties is complicated by the fact that many investigators believe that small lymphocytes are relatively undifferentiated cells and are capable of transforming into cells accepted as phagocytic macrophages.

It is questionable whether there is any significant difference in the phagocytic ability of polymorphonuclear neutrophiles arranged according to the Arneth or Schilling hemogram, except possibly in the case of the metamyelocyte. There have been a number of conflicting reports on the phagocytic activity of the neutrophiles based on the nuclear configuration by Block,³ Ponder and Flinn,⁴ Morita,⁵ Huddleson and Munser,⁶ and Strumia and Boerner.² Very little work has been done on the study of the phagocytic properties of the cells from leukemic blood. The im-

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in diameter. To separate vials was then added 0.1 cc. of a freshly prepared bacterial suspension from 24 hour broth cultures. The bacteria used as test particles consisted of *Staphylococcus aureus* and *Streptococcus viridans*. Both organisms were relatively non-virulent and were readily phagocytized. In order to compensate for the variability in the number of leukocytes the final suspension of bacteria was standardized to approximately five billion bacteria per cubic centimeter. This furnished an excess of bacteria in all experiments. If the total leukocyte count was over 100,000 cells per cmm., 0.15 cc. of the bacterial suspension was added. The vials were then tightly stoppered with corks which had been dipped in melted paraffin. The vials containing the leukocytes and bacteria were then rotated on a modified Robertson mixing wheel or agitator within the incubator at 37° for 10 minutes. The wheel was regulated to run at a constant speed of 16 revolutions per minute. At the end of 10 minutes the vials were removed and a drop of blood from each vial was used to make the usual blood smear. As a rule a differential count was made on 200 cells from both the staphylococcus- and the streptococcus-leukocyte mixtures, the smears having been stained by Wright's method followed by Giemsa's stain. The actual number of bacteria phagocytized in addition to the number of cells showing phagocytosis was noted. This afforded a quantitative as well as a qualitative measure of the phenomenon. In an effort to overcome the undetermined variations in the opsonic power of the patient's serum an agglutination test was carried out with the test organisms in each experiment. This would tend to make the results more reliable for comparative study.

Phagocytosis in Myelogenous Leukemia

This group included 10 cases, 8 of which were of the chronic variety. There was 1 case of acute myelogenous leukemia which showed a marked preponderance of myeloblasts. In the 10th case a diagnosis of Nägeli's monocytic leukemia was considered but because of the lack of sufficient differentiation toward mature monocytes it was diagnosed as a variety of subacute myelogenous leukemia.

The mature polymorphonuclear neutrophiles showed the greatest amount of phagocytosis as to both the number of bacteria ingested per cell and the per cent of cells engulfing bacteria. There

portance of this study lies in the fact that it might offer a physiological criterion for the separation of the cells of the various types of leukemia. Jacobsthal⁷ in 1921 studied the phagocytic activity of the cells from 1 case of chronic myelogenous leukemia and 1 of acute myeloblastic leukemia. He used cinnabar, staphylococci, and tubercle bacilli as test particles. In the case of chronic myelogenous leukemia he found the polymorphonuclear leukocytes normally phagocytic, but the myelocytes exhibited very little phagocytosis. In the acute myeloblastic leukemia the myeloblasts were actively phagocytic. Huddleson and Munser⁶ in 1936 studied a case of myelogenous leukemia, using *Brucella* organisms and immune serum. They found the greatest phagocytic activity in the mature polymorphonuclear leukocytes, less in the metamyelocytes, and no phagocytosis in the more immature forms. Strumia and Boerner² in 1937 reported the most complete study of the phagocytic properties of the circulating cells in the various forms of leukemia that has been published up to the present. Their material consisted of 11 cases of leukemia. In myelogenous leukemia they found occasional phagocytosis with all the immature granulocytes, including the myeloblast. However, the promyelocyte was the first cell showing definite phagocytic activity. In the 3 cases of lymphatic leukemia they found no evidence of phagocytosis in any of the lymphocytes, either mature or immature. In 2 cases of acute hemohistioblastic leukemia they found active phagocytosis by the hemohistioblast, monoblast and monocyte.

MATERIAL AND METHODS OF STUDY

The material used in this study consisted of blood from 17 patients with leukemia, 3 with infectious mononucleosis, 4 with lymphocytosis, and 2 with eosinophilia. The diagnosis in each instance was based on several hematological examinations and was confirmed by Dr. Hal Downey. The cells in all cases were classified according to the morphological criteria described by Downey.⁸

The experiments were performed as follows: The blood sample to be tested was obtained by venous puncture. Five to 10 cc. of blood were collected in a small pyrex flask containing 1 mg. of heparin in 0.1 cc. of saline for each 5 cc. of blood. One cc. of the heparinized blood was then placed in vials 6 cm. long and 1.5 cm.

locytes present. None of the immature lymphocytes showed any phagocytic activity. The lymphoid stem cell showed no difference when compared with the myeloblast of myelogenous leukemia. Definite phagocytosis was observed in an occasional small mature lymphocyte. The degree of phagocytosis increased as the cell grew larger with a corresponding increase in cytoplasm. The large leukocytoid lymphocyte of infectious mononucleosis showed the greatest phagocytic ability. However, the number of cells of the lymphoid series showing phagocytosis was very small as compared with the granulocytes.

Phagocytosis in Leukemic Reticuloendotheliosis

One case of leukemic reticuloendotheliosis was studied. The findings in this case were of special interest. A female, aged 23 years, had a white cell count of 75,600. Her illness lasted 7 weeks and her principal symptoms were fever, prostration and bleeding from the mucous membranes. The differential count showed 97 per cent large immature cells. There were a number of histoid stem cells or reticuloendothelial cells present. These cells possessed a moderate amount of basophilic stippled cytoplasm and a coarse sieve-like nuclear pattern. The majority of cells were histoid monoblasts. Their nuclear pattern indicated a reticuloendothelial origin. The lack of differentiation toward mature monocytes would not permit the diagnosis of Schilling's type of monocytic leukemia. An autopsy was performed by the author a few hours after the sample of blood was taken. The findings at autopsy were those of a leukemia with widespread hyperplasia of the reticuloendothelium of the liver, spleen, lymph nodes, thymus and bone marrow. The origin of the large monocytoid leukemic cells directly from the reticuloendothelium could be seen in many places, especially in the thymus.

Phagocytosis was observed in approximately 50 per cent of the histoid stem cells and 37 per cent of the histoid monoblasts. This was the greatest amount of phagocytosis observed in any of the undifferentiated cells studied.

Phagocytosis in Eosinophilia

Two cases of eosinophilia were studied. The 1st case had a 12 per cent eosinophilia. The clinical diagnosis, which was confirmed

was a small but definite decrease in the degree of phagocytosis by the metamyelocytes or non-filamented forms of polymorphonuclear neutrophils when compared with the mature neutrophilic or filamented forms. The phagocytic activity of the myelocytes, promyelocytes, leukoblasts and myeloblasts appears to be directly proportional to the maturity of the cell. There was a marked decrease in phagocytosis in the more immature forms. There was no correlation between the degree of phagocytosis observed and the relative amount of cytoplasm of these cells, except in the case of the myeloblast. This latter cell has a small amount of cytoplasm and less than 1 per cent showed any phagocytic activity. The eosinophilic metamyelocytes and myelocytes were less phagocytic than the neutrophilic variety of these cells. The mature monocytes were actively phagocytic but the immature monocyte or monoblast showed very little. No definite phagocytosis was observed in any of the basophiles but allowance must be made for the fact that it is difficult to differentiate between the basophilic granules and the bacteria.

Phagocytosis in Mixed Leukemia

One case of mixed leukemia was studied. The total white count was 19,800. The differential count showed the unusual picture of myeloblasts and developmental stages leading to granulocytes on the one hand, and to lymphocytes on the other. Active phagocytosis was observed in the polymorphonuclear neutrophils. The metamyelocytes and myelocytes showed moderate phagocytosis. No organisms were found to be ingested by any of the more immature granulocytes. None of the numerous lymphocytes, regardless of size or maturity, showed any phagocytic activity except that one large lymphocyte contained fourteen staphylococci and one small lymphocyte contained one staphylococcus within its cytoplasm.

Phagocytosis in Lymphatic Leukemia, Lymphocytosis and Infectious Mononucleosis

This group included 4 cases of chronic lymphatic leukemia, 1 case of acute lymphatic leukemia, 4 cases of absolute or relative lymphocytosis, and 3 cases of infectious mononucleosis. Active phagocytosis was observed in the small number of mature granu-

phagocytosis increased as the lymphocyte grew larger with a corresponding increase in cytoplasm. The leukocytoid lymphocyte of infectious mononucleosis showed the greatest phagocytic activity. However, the number of cells of the lymphoid series showing phagocytic activity was very small as compared with the granulo-

TABLE I
Summary of Data from 26 Experiments

	Number of cells	Number with bacteria	Percentage	Average number of bacteria per cell
Polymorphonuclear neutrophiles	1478	1363	92.0	17.90
Basophiles	150	0		
Eosinophiles	224	181	84.80	11.19
Metamyelocytes	387	263	67.0	15.30
Eosinophilic metamyelocytes	56	22	39.27	10.17
Myelocytes	484	197	40.0	5.90
Eosinophilic myelocytes	69	25	36.21	7.84
Promyelocytes	370	40	10.80	4.52
Leukoblasts	487	14	2.80	5.07
Myeloblasts	659	1	0.15	1.0
Histoid stem cells (reticuloendothelial)	30	14	46.66	6.21
Monocytes	150	114	76.0	16.09
Monoblasts	115	5	4.35	6.80
Histoid monoblasts (reticuloendothelial)	141	53	37.69	3.89
Lymphoid stem cells (lymphatic leukemia)	15	0		
Immature lymphocytes	267	0		
Small lymphocytes	1616	19	1.17	2.63
Mesolymphocytes	1068	22	2.06	6.27
Large lymphocytes	604	45	7.45	4.44
Leukocytoid lymphocytes	408	49	12.0	11.44
Plasma cells	10	0		
Reider lymphocytes	1	1		2.0

cytes. Downey⁹ states that the small lymphocyte is a relatively undifferentiated cell. The work of Maximow,¹⁰ Bloom,¹¹ Parker and Rhoads,¹² and Pierce,¹³ would indicate that the small lymphocyte is a cell of various potencies and is capable of enlarging and differentiating toward phagocytic macrophages. Hence one can conclude from the findings in this study that lymphocytes, even in their prephagocytic stage, may occasionally show some phagocytic

later at autopsy, was periarteritis nodosa. The 2nd case was a 75 per cent eosinophilia of undetermined origin. Definite phagocytosis was observed on the part of the eosinophiles. However, both the percentage of eosinophiles showing phagocytosis and the number of organisms ingested per cell were less than in the polymorphonuclear neutrophiles.

DISCUSSION

The 26 experiments covered a wide range of both mature and immature cells. No correlation was observed between the percentage of cells showing phagocytosis or the number of organisms ingested and the agglutination titer of the serum. However, the test organisms were relatively non-virulent because of long laboratory passage. Little difference was noted in the degree of phagocytosis as shown by the cell toward either of the two test organisms. In Table I the results obtained in all of the 26 experiments are combined. This should afford a sufficient number of cells of the various types to allow a fairly accurate determination of the percentage of cells showing phagocytosis with the average number of both staphylococci and streptococci per cell.

An analysis of Table I shows that the mature polymorphonuclear neutrophiles exhibited the greatest phagocytic ability. The monocytes, eosinophiles and metamyelocytes were also actively phagocytic. The phagocytic activity of the myelocytes, promyelocytes, leukoblasts and myeloblasts appears to be directly proportional to the maturity of the cell as there was a marked decrease in phagocytosis in the more immature forms. An exception was found in the histoid stem cell and histoid monoblast of leukemic reticuloendotheliosis as these immature cells arising directly from the reticuloendothelium were actively phagocytic. The histoid monoblasts in this condition differed markedly in their phagocytic activity from the monoblast arising from the myeloblast of myelogenous leukemia. The phagocytic ability of these immature cells clearly separates leukemic reticuloendotheliosis from other types of leukemia, including the Nägeli type of monocytic leukemia in which the myeloblast is the stem cell.

The findings in the lymphocytic series were not in accord with those of Strumia and Boerner.² These authors state emphatically that lymphocytic cells never show lymphocytes. The degree of

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activity under experimental conditions. This tendency appears more marked in the leukocytoïd lymphocyte of infectious mononucleosis.

These experiments would indicate that phagocytosis is a physiological process not confined to any one type of cell. Certain cells such as eosinophiles and lymphocytes which ordinarily show no phagocytic activity may under experimental conditions become phagocytic. The maturity of the cell is an important factor in determining its phagocytic ability.

SUMMARY AND CONCLUSIONS

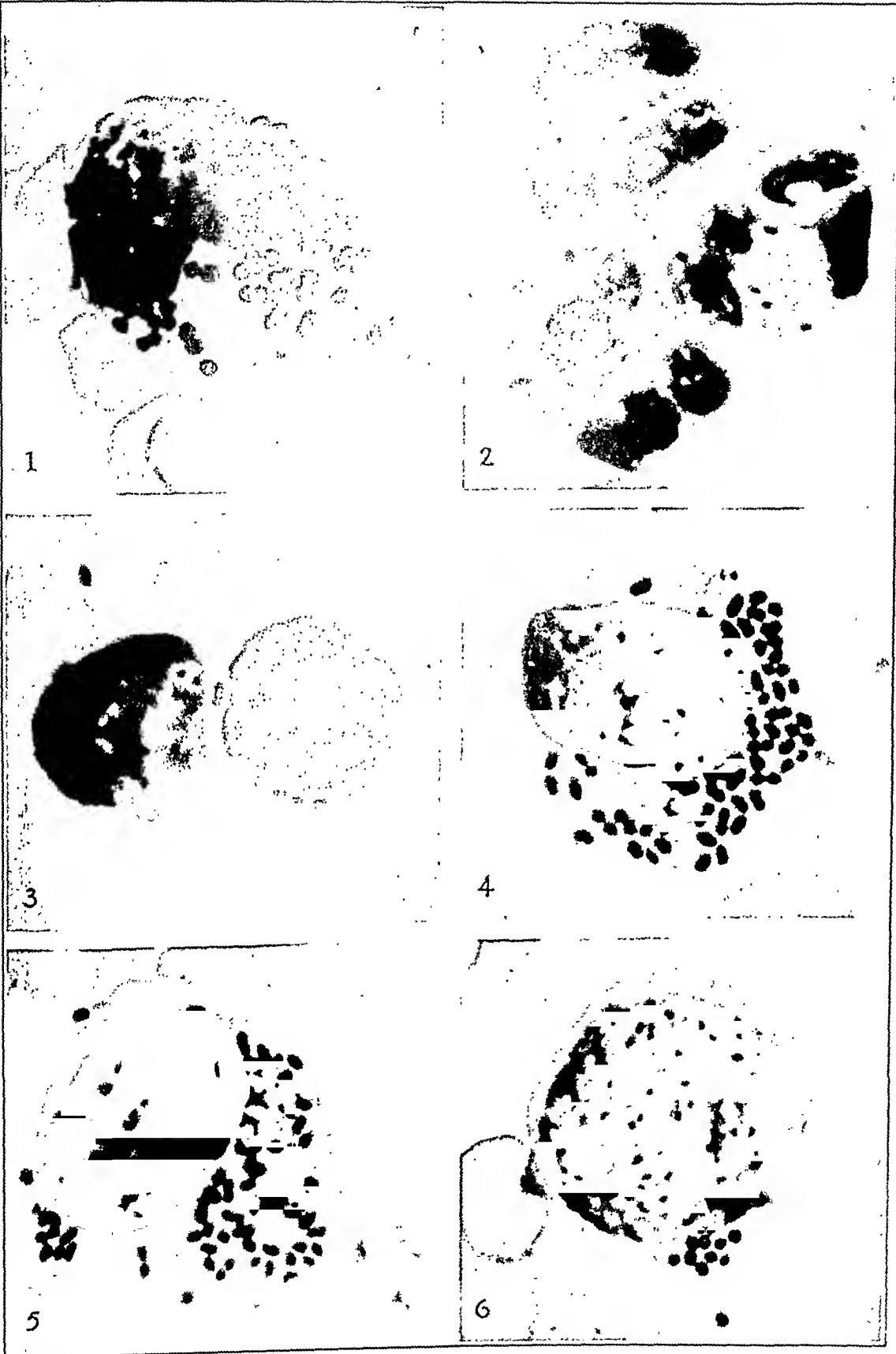
1. The phagocytic activity of the leukocytes from 17 cases of leukemia, 3 cases of infectious mononucleosis, 4 cases of lymphocytosis, and 2 cases of eosinophilia were investigated. Staphylococci and streptococci were used as test particles.

2. The mature polymorphonuclear neutrophiles showed the greatest amount of phagocytosis both as to the number of bacteria ingested per cell and as to the percentage of cells engulfing bacteria. The monocytes, eosinophiles and metamyelocytes were also actively phagocytic.

3. The phagocytic activity of the myelocytes, promyelocytes, leukoblasts and myeloblasts appears to be directly proportional to the maturity of the cell as there was a marked decrease in phagocytosis in the more immature forms.

4. An exception was found in the histoid stem cell and histoid monoblast of leukemic reticuloendotheliosis as these immature cells showed an unusual degree of phagocytosis.

5. Phagocytosis was observed in a small per cent of mature lymphocytes. The degree of phagocytosis increased as the cell grew larger with a corresponding increase in cytoplasm. The leukocytoïd lymphocyte of infectious mononucleosis showed the greatest phagocytic activity. Hence, lymphocytes in their pre-phagocytic stage may occasionally show phagocytosis under experimental conditions.



DESCRIPTION OF PLATE

PLATE 138

- FIG. 1. Mature monocyte from myelogenous leukemia showing phagocytosis of staphylococci. $\times 1800$.
- FIG. 2. Eosinophiles from eosinophilia showing phagocytosis of streptococci. $\times 1500$.
- FIG. 3. Small mature lymphocyte from lymphatic leukemia showing phagocytosis of streptococci. $\times 1500$.
- FIG. 4. Large mature lymphocyte from infectious mononucleosis showing phagocytosis of streptococci. $\times 1800$.
- FIG. 5. Leukocytoid lymphocyte from infectious mononucleosis showing phagocytosis of streptococci. $\times 1800$.
- FIG. 6. Histoid stem cell from leukemic reticuloendotheliosis showing phagocytosis of staphylococci. $\times 1800$.

LYMPH NODE METASTASIS OF SARCOMA *

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INTRODUCTION

A few instances of metastasis of sarcoma by lymphatics, preceding or even with evidence of blood-borne metastasis, occurring among our cases have raised the question of the importance of this method of dissemination. Because the completeness with which various groups of cases are presented in the literature varies so greatly, it is impossible to draw any conclusion as to the frequency of lymphatic metastasis by considering the cases here reviewed as a whole. Many reports of sarcoma were examined in the hope that there might be mention of lymphatic metastasis; often simply the death of the patient was recorded without specific mention of the presence or absence of metastasis. However, a few large series by individual authors mention metastasis specifically.

REVIEW OF THE LITERATURE

Küttner ¹ reported 740 cases of sarcoma, including myelogenous sarcoma and lymphosarcoma but not melanoma, 326 of which had been followed, and stated: "Die regionären Drüsen sind prinzipiell zu entfernen, da von 132 Metastasen 79 auf dem Lymphwege erfolgten." The high incidence of lymphatic metastasis in this series is undoubtedly due to the inclusion of lymphosarcoma and leukemia, which vitiates the figures.

Seitz and Wintz ² reported 16 cases of sarcoma, 1 a melanoma. In but 1 case (Case 1) was lymph node metastasis proved; in 2 additional cases (Case 4 and Case 13) the primary sarcoma was proved and lymph node metastasis probable; in 2 more lymph node metastasis was probable but the nature of the primary tumor was uncertain.

Seyerlein and Hölzel ³ wrote: "Wir halten gerade bei Sarkom die

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mention is made of metastasis, 5 showing lymph node involvement. In 1 of these disarticulation of a finger, because of a polymorpho-cellular sarcoma, was followed a year later by axillary node metastasis; death occurred in 3 months in spite of radiation. Another patient presented an inoperable polymorphocellular sarcoma of the skin of the abdomen with massive lymph node and cutaneous metastases; death occurred within the year. A sarcoma of the leg was removed from another patient by operation and the inguinal node metastases radiated; a year later there was recurrence in the inguinal nodes and the patient died 14 days after admission to the hospital. A patient with an inoperable spindle cell sarcoma of

TABLE I
(Adapted from *Leucutia* ⁷)

Number	Histological diagnosis	Origin	Extent of metastases
8	Leiomyofibrosarcoma	Aortic wall	Abdominal nodes
43	Angiosarcoma	Sclerae	Later metastases to lymph nodes and chest
57	Myxosarcoma	Abdominal wall	Abdomen, inguinal nodes
66	Large spindle cell sarcoma	Neck	Lymph nodes
83	Fibrosarcoma	Finger	Axillary nodes
88	Spindle cell sarcoma	Thigh	Distant lymph nodes
32	Spindle cell sarcoma	Breast	Regional nodes and mediastinum
16	Spindle cell sarcoma	Maxilla	Entire face, nodes and neck
92	Spindle cell sarcoma	Sole	Lymph nodes, skin and lungs
67	Spindle cell sarcoma	Thigh	Mediastinum

the foot with inguinal node metastases, operated on and radiated, died a year later. Another individual with a round cell sarcoma of the ileocecal region with large nodal metastases died 3 months after resection. (This was probably some form of lymphoma.) For the sake of completeness it may be mentioned that Meves also lists 19 cases of sarcoma of the glandular organs, in none of which is there specific mention of lymphatic involvement. He further reports 2 cases of lymph node involvement (1 microscopically proved) which are not included in the list.

Leucutia ⁷ in a tabulation of 101 sarcomas of the soft parts includes the above cases with lymph node involvement. (We have omitted his "round cell" sarcoma.)

prophylaktische Bestrahlung für angezeigt, . . . weil wir wissen, dass das Sarkom sich nicht allzu selten auf dem Wege der Gewebsspalten und Lymphbahnen ausbreitet." Omitting 7 cases diagnosed as lymphosarcoma, and 3 of melanoma (1 of them so reported; in 2 others a dark tumor is mentioned), 60 cases of sarcoma were reported by the authors. There was swelling of the lymph nodes in 8 cases with microscopically proved tumors, and in 2 additional cases also where there was no microscopic examination. Of the 8 cases 4 were polymorphocellular, 1 polymorpho- and spindle celled, 1 round celled, and 2 were not specifically mentioned. In only 1 case, that of round celled sarcoma, was microscopic examination of the lymph nodes specifically mentioned. None of these cases is described as that of fibrosarcoma.

Among 69 cases of "giant cell sarcoma of bone" Coley⁴ mentions 1 case with lymphatic metastasis. A giant cell sarcoma of the lower third of the femur was treated by curettage followed by amputation. The patient remained well for 11 months and then developed metastases to the iliac lymph nodes and lung, which proved fatal. The fact that lung metastases may remain asymptomatic for relatively long periods makes it impossible to determine with the data available whether the lung metastases arose from the original tumor or from the lymphatic involvement.

Schreiner⁵ reports one lymph node metastasis among 8 cases. Following removal of a uterine "myoma malignum" there was recurrence with metastases to the mesentery, aortic lymph nodes and ribs. He includes a microphotograph of one of the metastatic nodules removed at autopsy. In response to our inquiry Dr. Schreiner very kindly reviewed the slide from which the microphotograph was made, also the autopsy notes, and writes that it was believed to be from a mesenteric lymph node.

Meves⁶ reports a series of cases in which are included 59 osteogenic sarcomas, 8 of which are specifically mentioned as having metastases, 2 of them in inguinal lymph nodes; 1 of these was a polymorphic spindle cell sarcoma of the tibia, the other was a large celled, mixed cell sarcoma of the calcaneus. The first of these 2 patients died during treatment; amputation, radiation and removal of the inguinal nodes in the second patient were followed by death 1 year and 2 months later. In this same series are included 45 cases of sarcoma of the soft parts, in 14 of which specific

malignant tumors of bone." Further, "Spreading of tumor metastases through lymphatics is infrequent. It is obvious that a metastatic involvement of regional lymph nodes in malignant bone tumors means more as an indication of generalization than in carcinoma. An occasional simple lymphadenitis in malignant bone tumors should be clearly distinguished from a metastatic involvement of the nodes. Such a lymphadenitis is usually found following an infection after ulceration of the tumor or after an exploratory incision. However, an inflammatory reaction in the regional lymph

TABLE II
Frequency of Lymph Node Metastases of Sarcoma

Author	Total number of cases of sarcoma (exclusive of lymphosarcoma)	Cases with lymph node metastasis	Per cent of lymph node metastasis
Seitz and Wintz ²	15	5	33.3
Seyerlein and Hölzel ³	60	8	13.3
Coley ⁴	69	1	1.4
Schreiner ⁵	8	1	12.5
Meves ⁶	123	7	5.6
Leucutia ⁷	101	10	9.9
Thibaudeau and Kress ⁸	42	2	4.8
Meyerding, Broders and Hargrave ⁹	152	5	3.3
Willis ¹⁰	14	2	14.0
Total	584	41	7.0

nodes is also encountered when there is no ulceration present."

Among scattered cases of sarcoma metastatic to lymph nodes may be mentioned 2 reported by Coley.¹² A 48 year old female (Case 18) had a spindle celled sarcoma of the left thigh with evidence of involvement of the inguinal nodes. Case 19 was that of a 29 year old male who after removal of a tumor of the spermatic cord had speedy metastasis of a round celled sarcoma (probably embryonal carcinoma) in the inguinal nodes.

In an extensive review of tumors of the heart, including about 40 cases of primary sarcoma, Goldstein¹³ cites a report by Escher (1909) of round cell sarcoma of the heart with metastasis in tracheal lymph nodes and suprarenal in a 72 year old female. Omitting carcinoma, lymphosarcoma and melanoma, there is only 1 other case cited from the literature in which lymph node me-

It will be noticed that in the 10 cases listed 1 is included in which there is mention of mediastinal metastasis only, with no mention of lymph nodes; it is included because of the likelihood that this metastasis was actually to the mediastinal nodes. Of the 101 cases, some of which must have been lymphoma, 42 showed metastases; its occurrence was not known in 6. Of the cases with metastases almost one-fourth had lymph node metastasis, and one-tenth had lymph node metastasis only. The fact that 2 cases with lymph node metastasis survived 6 and 5.9 years emphasizes the lack of specific mention of histological proof of the metastasis.

Thibaudeau and Kress⁸ found in 42 cases of myxosarcoma 2 with metastasis to the regional nodes.

Meyerding, Broders and Hargrave⁹ report: "Invasion of regional or distant lymph nodes is an occasional feature of fibrosarcoma. Mediastinal, bronchial, and retroperitoneal lymph nodes are at times involved. . . . Of the cases in this series (152 with available pathologic specimens, diagnosed fibrosarcoma, of which 138 had complete histories) there were five instances of involvement of regional lymph nodes; two of these cases involved the axillary, and three the inguinal lymph nodes."

Willis¹⁰ includes a large group of reported cases of lymph node metastases of rhabdomyosarcoma, leiomyosarcoma, bone sarcoma, myxoliposarcoma, and malignant synovial tumors, citing 33 references in all. He reports 2 such cases from his own group of 14 sarcomas. A 55 year old female (Case 218, reported by King) had a toe amputated for tendon sheath synovial sarcoma; within a few months enlarged inguinal nodes appeared and progressed to a large ulcerated tumor in spite of X-ray therapy. Autopsy 20 months later showed tumor deposits in the iliac and lumbar nodes but no visceral metastases. The diagnosis was: "Highly anaplastic polymorphic and giant-celled growth quite unlike the recognizably synovial neoplasm in the amputated toe." At autopsy of a 21 year old male a large round celled sarcoma of the soft tissues of the supraspinous fossa of the scapula was found to have metastasized to the lungs, mediastinal lymph nodes, ribs, many vertebrae and base of the skull.

Kolodny¹¹ states: "The idea that the blood stream is the only path of spreading of metastases in sarcoma does not hold true in

Mallory²² reports a case of "leiomyosarcoma of the distal third of the esophagus with extension into and ulceration of the mediastinal lymph nodes. . . . The tumor had spread through the esophageal wall and involved a group of mediastinal lymph nodes on the anterior surface of the esophagus just behind the pericardium which were greatly enlarged to a mass six or seven centimeters in diameter."

McFarland²³ states: "Metastasis may take place through the blood or lymph and may occur in the usual distribution — liver, lungs, lymph nodes — but sometimes, as in Wainwright's case, the distribution is perplexing." In Wainwright's case there was metastasis in the neck (not specifically mentioned as occurring in the lymphatics; a microphotograph is included) and, he states, "Although there were two other metastatic tumors removed surgically, the patient was alive twelve years after hysterectomy." He also states: "One of Dr. Tracy Mallory's patients, in the Massachusetts General Hospital, had a primary nodular tumor of the duodenum with invasion of the regional lymph nodes." No reference is given for either of these cases.

Melnick²⁴ reported generalized primary angiosarcomatosis of the lymph nodes in a 64 year old white male. Biopsies of four different lymph nodes revealed angioblastomatous tumor, fairly well encapsulated, and "apparently independent of the lymphatic tissue or the reticulo-endothelial apparatus."

Charache²⁵ reports the case of a 44 year old male who hit his thigh; a week later a tumor appeared, which was removed 6 months later at another hospital. In spite of X-ray treatment the wound did not heal. Autopsy 3 weeks after the patient was seen revealed a "rhabdomyosarcoma of the left thigh with metastases to the lungs, pleura, pericardium, liver, kidneys, and inguinal, tracheal, aortic lymph nodes, skin."

Elmer and Boylan²⁶ report a sarcoma arising after a fracture of the neck of the femur in a 52 year old male and state: "Evidences of new growth were found in the lungs, liver, lymph glands, ribs, and cervical vertebra. These findings pointed to a mixed chondroma such as a myxochondrosarcoma."

Rehbock and Hauser²⁷ report a case (Case 1) that they regarded as liposarcoma which involved lymphatics with metastases to lymph nodes as well as generalized metastases.

tastasis is mentioned. He states further: "Perlstein reports an interesting case of mediastinal mixed-celled sarcoma, affecting the pericardium. . . . There were metastases in the pleura and mediastinal lymph nodes."

Antonow¹⁴ presents the case of a 21 year old female showing at operation a nodular tumor, about 7 cm. in diameter, involving the stomach, with nodules in the gastrocolic ligament and duodenum. The tumor extended to the tail of the pancreas and the retroperitoneal tissue. The regional nodes, removed with the leiomyosarcoma, were proved histologically to be involved. D'Aunoy and Zoeller¹⁵ reviewed the literature of sarcoma of the stomach and added 4 cases, of which 2 showed lymph node metastasis, only 1 histologically proved (sarcoma). Both these cases suggest lymphoma.

In Cohen's¹⁶ case, "Necropsy revealed a primary leiomyosarcoma of the left kidney with metastases to both lungs, right kidney, suprarenal glands, ileum, mediastinal and mesenteric lymph nodes and brain."

Raiford,¹⁷ while describing tumors of the small intestine, writes: "These sarcomas (of the small intestine) do not metastasize as readily as carcinomas. When metastasis does occur, the mesenteric glands are most frequently involved first, after which secondary invasion may reach the liver or lungs." He gives no evidence for this statement.

Greenblatt¹⁸ reports the case of a 74 year old female who had carcinosarcoma in the skin of the left breast. Two months after local excision swelling appeared in the left axilla. Two somewhat enlarged lymph nodes were removed and diagnosed as fibrosarcoma. Husted¹⁹ reports the case of a breast tumor, first diagnosed as fibro-adenoma. It recurred and was removed twice, finally metastasizing to the axillary and supraclavicular nodes; biopsy of the nodes showed a round cell sarcoma.

Dyke²⁰ reports a case of giant cell tumor of the patella and femur with metastases to the mediastinal and peritoneal lymph nodes, scalp, lungs and kidneys. At autopsy the metastases showed necrotic and fibrous tissue with foci of giant cells.

Hall²¹ reports the case of a malignant hemangioma of the lung in a 40 year old female who died following hemorrhage into the right pleural cavity. Lymph node metastases were found.

In 8 of the cases, in addition to removal of the tumor at the original site, there was excision of lymph node or nodes without radical dissection, followed by either radium or X-ray in 3. One of the non-irradiated patients survived for 8 months (X-ray was tried terminally) and the other is still alive. The others died within 4 months, 3 months, and 2 months.

In the 14 cases with surgical removal of lymph nodes, those on whom a radical dissection was done survived for a longer period than those on whom there was simple excision of the involved node. Two of 5 individuals are alive (for 6 years and 23 months respectively), and the other 3 who died survived an average of 11 months after removal of involved nodes; as compared with 3 out of 8 alive and an average survival period of about 4 months for the dead. Of the 5 patients on whom a radical dissection of lymphatics was done, the lesion was in an extremity in 2, while in the 8 in which simple excision was done the tumor arose in an extremity in 3 cases. Exclusive of the single case of amputation in each group (both individuals died) there were no recurrences in the group with radical dissection, and 2 cases of recurrence in the group with excision of the involved lymph node only. In the first group the number of operative interferences before the patient was seen at the hospital were 3, 3, 2, 1 and 0; while in the latter group they were 14, 2, 3, 1, 1, 1, 0 and 0.

One additional case of fibrosarcoma metastatic to the lymph nodes has been sent to this laboratory for pathological diagnosis (36-1183); it has not been included among our own hospital cases but is mentioned here.* A 48 year old female complained of uterine bleeding at intervals for 6 months, pain and progressive swelling of the abdomen, and frequency. The abdomen was found to be of a size consistent with a 7 months pregnancy, and vaginal examination showed a large mass thought to be the uterus. Laparotomy revealed a hard, nodular, somewhat friable, very vascular solid tumor about 25 cm. in diameter, with the transverse colon attached over the anterior surface. Exploration resulted in considerable bleeding. The origin of the tumor seemed to be from the retroperitoneal space and there was no apparent attachment to the tubes or ovaries; removal was impossible, but a lymph node

* We wish to thank the Sturdy Memorial Hospital, Attleboro, from which the specimen was received, for details of the history.

The 19 year old male patient of Fayein's²⁸ had right axillary and subclavian adenopathy which regressed with radiation, but metastases to the right arm and breast and neurological symptoms in both legs followed. He became dyspneic and died. The histological interpretation was considered questionable, but carcinoma was ruled out and malignant melanoma was thought unlikely. The case is described as one of polymorphous sarcoma with multiple metastases. No mention is made of the possibility of lymphoma.

REVIEW OF OUR CASES

Including fibrosarcoma, myxosarcoma, liposarcoma, osteogenic sarcoma, definitely malignant leiomyosarcoma, and rhabdomyosarcoma, but excluding lymphosarcoma, carcinosarcoma, sarcoma of endometrial stroma, and melanotic sarcoma, there are in a total of 237 sarcomas we have studied 17, or 7 per cent, which have been proved to have metastasized to lymph nodes. All but 3 of these were proved before death. There were in addition 5 cases in which the clinical diagnosis specifically included metastasis to lymph nodes, and 3 others in which it included metastasis to the groin. The cases with histologically proved lymph node metastasis are summarized in Table III.

Of the 17 patients with lymph node metastasis only 5 are living, 1 for 6 years since radical removal of a chest tumor (first recurrence) and involved axillary glands (he has recently developed a palpable liver); 1 for 2 years following radical dissection of iliac glands, 3 months after the third removal of a tumor of the leg; another for 2 weeks after removal of a metastatic inguinal node; 1 for 2 weeks following removal of a cervical node after three recurrences of the nasopharyngeal primary lesion; and the 5th 19 months since excision of a submaxillary node showing fibrosarcoma (a lesion on the lip was excised 6 months previously and pathological examination showed no evidence of malignancy).

A radical dissection for removal of lymph nodes was done in 5 cases; 2 patients are living and 3 are dead. In 1 of the latter a groin dissection was done 2 months after the fourth removal of a tumor over the sacrum; the patient died 4 months later. In another a forearm amputation combined with dissection of the axilla was the first operation, but the patient died a year later. In the last, radical mastectomy permitted survival for 1½ years.

NEDH. 77543	37	M	Fibrosarcoma	Simple ex- cision	Pain in left leg for 1½ yrs. Ulcer 12 by 8 cm. on posterior thigh; semicartilagi- nous base	Clinical diagnosis car- cinoma of lip. V excision. Diagnosis chronic inflammation	6 mos. later, hard lymph node below left jaw excised. Diagno- sis fibrosarcoma with metastasis in lymph node	8 mos. after first op- eration thickened area on lip. Second V excision. Diagnosis chronic inflammation	?	25 mos.	19 mos.	Living
Pondville	42	M	Fibrosarcoma	Simple ex- cision		Apparently complete excision of tumor	Nodule appeared in left groin 3 mos. after operation. Ex- cised elsewhere 10 mos. later. Diagnosis sarcoma. 6 mos. later more nodules which grew and had been ulcerated for some time when admitted to hospital. X-ray showed metastasis to lung. X-ray treat- ment groin	Autopsy showed fibro- sarcoma with metas- tasis to lung and regional lymph node	4 yrs.	2 yrs. 4 mos.	2 yrs.	Dead 3 mos. later
1111-11-30n W & S 47	54	M	Fibrosarcoma	Simple ex- cision	Lesion of right eyelid recurred 6 yrs. after radium; increased in size of radium and electric needle	Excision upper eyelid. Diagnosis fibrosar- coma. Recurred 2 mos. later. Mass 4 by 6 cm. removed	2½ mos. after first operation overlying right parotid excision. Diagnosis fibrosar- coma, metastatic	2¼ mos. after opera- tion nodule removed from edge of orbit. Diagnosis fibrosar- coma. This recurred and a gland appeared in the submaxillary region	8½ yrs.	5¼ mos.	3 mos.	Dead
Palmer 37-964	59	F	Fibrosarcoma	Simple ex- cision	Lump removed from right leg. Recurred in 9 mos. with ex- cision and X-ray. Re- curred 1 yr. and 5 mos. ago. Excision and X-ray	Excision of recurrence and of iliac nodes	2 yrs. and 2 mos. from onset nodule in right inguinal region. Sur- gical excision shows one of four nodes involved	6 mos. later lung me- tastasis. At autopsy metastasis to lung, pleum, liver, adrenal, vertebra, lymph node	2 yrs. 3 mos.	2 yrs. +	2 wks.	Alive and well
Palmer 35-1311	63	M	Fibro-sarcoma	Simple ex- cision	5 yrs. ago roughening of skin over rectus. Nodule removed 14 and again 7 mos. ago. Recurrence. Diagno- sis said to be fibro- sarcoma	Excision of recurrence. Diagnosis fibrosar- coma	3 wks. later large right axillary mass excised. Diagnosis fibrosar- coma with metastasis to lymph node	6 mos. later lung me- tastasis. At autopsy metastasis to lung, pleum, liver, adrenal, vertebra, lymph node	5 yrs. 9 mos.	8¼ mos.	8 mos.	Dead
1111-15-14	67	F	Leiomyo- sarcoma	Simple ex- cision	Fell 8 wks. ago. 6 wks. ago growing painful mass in right buttock. Mass in ischio-rectal fossa and another in pelvis	Operation at NEDH tumor of sigmoid 7.5 cm. in diameter. Di- agnosis leiomyosar- coma. Perineal resec- tion of bowel and ischio-rectal mass. Di- agnosis leiomyosar- coma	6 mos. after first seen lump 2 cm. in diam- eter in left axilla. Excision well outside its border. Diagnosis leiomyosarcoma, me- tastasis in lymph node	13 mos. after admis- sion. No recurrence	17 mos.	15 mos.	8 mos.	Dead 10 wks. later

* Palmer refers to the report by Dahland.

† W & S refers to the report of Watten and Sommer.

TABLE III

Summarized Case Reports

Case No.	Age	Sex	Microscopic diagnosis	Nature of operation	Course before admission	Condition on admission and treatment	Time of discovery of lymph node metastasis	Subsequent course	Years since			Result
									Onset	Treatment	Recognized lymph node metastasis	
Pondville 12682 37A150	23 yrs.	F	Osteogenic sarcoma	Biopsy	1 yr. ago lump in left hip near vertebral column. Partial excision 10 mos. ago. Diagnosis osteogenic sarcoma. Became pregnant. Recurrence 6 mos. ago; swelling size of fooball noted at time of Cesarean delivery 2½ mos. ago. Cordotomy 2 mos. ago; paralysis	X-ray showed extensive bone tumor in left side of pelvis and fluid in left chest. Symptomatic treatment	At autopsy. Diagnosis osteogenic sarcoma involving lymph nodes, lungs, lumbar vertebrae		15 mos.	—	o	Dead
Pondville 231 Daland *	31	M	Kaposi sarcoma	Biopsy	Epigastric distress(?) 1 yr. 5 mos. ago brown skin nodules abdomen and groin; 4½ mos. ago on mouth	Scattered skin nodules and generalized adenopathy. Biopsy chest wall and ear. Excision buccal tumors	At autopsy. Section of axillary and inguinal lymph nodes showed many areas of hemorrhagic tumor tissue which was like the tumor elsewhere		1 yr.?	4 mos.	o	Dead
Pondville 11468	44	F	Retropertitoneal sarcoma	Biopsy	5 yrs. ago hysterectomy and removal of a tumor elsewhere; at same hospital 2 yrs. ago lung metastasis seen by X-ray. Biopsy nodule left neck showed metastatic carcinoma	Large mass in abdomen	When first seen biopsy mass in left axilla. Diagnosis carcinoma of lymph node	Abdominal mass increased. At autopsy nodes replaced by tumor tissue. Diagnosis retroperitoneal sarcoma of kidney	5 yrs.	2 yrs.	25 mos.	Dead
HH-28-1479	72	M	Fibrosarcoma	Biopsy	Dysphagia	Ulceration right tongue. Biopsy right submaxillary lymph node showed fibrosarcoma	When first seen	Radium seeds in tongue. X-ray of chest showed temporary improvement	8 mos.	4 mos.	4 mos.	Dead
Palmer 37-1570	6	M	Rhabdomyosarcoma	Simple excision	1 mo. gradual nasal obstruction. Excision of palm-sized mass from nasopharynx. Deep X-ray therapy. Recurrence in 1 mo. Partial excision	Extensive nasopharyngeal tumor. Complete removal followed by implantation of radium needles. Recurrence in 7 wks. Implantation of radium needles	3 wks. later node 4.5 by 2.5 by 2.5 cm. removed from right neck. Diagnosis metastatic rhabdomyosarcoma. Large nodes also present on left side of neck		8 mos.	7 mos.	2 wks.	Alive, tumor present
HH-19-860 † W & S 12	13	M	Fibromyxosarcoma	Amputation. Simple excision of node	Growth on left hand present at birth. Removed 14 times, 3 in last year. Diagnosis fibromyxosarcoma	Intermittent improvement with radium. 2 yrs. later persistent ulceration and lymph node in axilla 2 cm. in diameter	Amputation and removal of lymph node; radium seeds to adjacent lymph node. Diagnosis (hand and lymph node) sarcoma	4 mos. later rapidly growing masses in axilla and over end of spine	15 yrs.?	2½ yrs.?	4 mos.	Dead

was removed. The patient died 12 hours later. At autopsy the biopsied lymph node was replaced by fibrosarcoma.

DISCUSSION

Lymphatic metastasis of sarcomas (exclusive of melanosarcoma and lymphosarcoma and related forms) has been recognized by many, but few references have been made to prophylactic dissection of lymph nodes. The incidence of lymphatic metastasis of the sarcomas here presented agrees roughly with the average incidence of such metastases (7 per cent) among the reported groups of cases that have been reviewed, being 7 per cent (histologically proved cases) and probably 10 per cent (clinical evidence included) in this series. The importance of treating such metastasis is proved by the fact that in one instance the patient is still living 6 years after removal of involved nodes; in another with a 2 year survival period the proximal nodes were negative and the more distant ones showed metastasis. Both groups of nodes were removed 2 months after removal of the last recurrence. In contrast with the latter case is one in which lymph node involvement was noted 3 months after excision of the tumor; the patient finally had the metastatic mass excised 10 months after he first noticed it; it recurred in 6 months, and it was not until another 6 months that he came to the hospital with an ulcerated mass in the groin and lung metastases. He died 3 months later from local hemorrhage. In this case, in spite of the fact that the lung metastasis must have progressed slowly, since it was not the cause of death 3 months after being recognized, the lymphatic metastasis almost certainly preceded it. Had a prophylactic dissection been performed, the course of the disease might possibly have been altered. The 3 cases in which lymph node metastasis was proved only at postmortem examination would seem to confirm Kolodny's statement that such metastasis is a more serious prognostic sign than that from carcinoma.

Even though the number of cases be too small to suggest that lymphatic dissection be done coincident with every excision, it does seem to allow the interpretation that such dissection should be done whenever feasible if an operation as serious as amputation or radical excision is being considered.

TABLE III (Continued)

Case No.	Age	Sex	Microscopic diagnosis	Nature of operation	Course before admission	Condition on admission and treatment	Time of discovery of lymph node metastasis	Subsequent course	Years since			Result
									Onset	Treatment	Recognized lymph node metastasis	
III-35-675	37 1/2	F	Fibrosarcoma	Radical excision	Ball hit thigh 16 mos. ago. 2 mos. later slowly increasing lump, excised elsewhere 11 mos. ago. Recurred 2 mos. ago. Diagnosis said to be sarcoma of low malignancy	Wide excision subcutaneous mass 2 cm. in diameter. Diagnosis fibrosarcoma	3 3/4 mos. after operation chain of hard saphenous glands up to 1.5 cm. in diameter but none in inguinal region. Radical groin dissection. Diagnosis highest (ilac) lymph nodes negative; inguinal and saphenous lymph nodes show metastatic fibrosarcoma	No evidence of disease	3 yrs. 8 mos.	2 yrs. 4 mos.	1 yr. 11 mos.	Living
III-31-610 W & S 20	28	F	Fibrosarcoma	Radical excision	Tumor of back removed 3 yrs., 2 1/2 yrs., and 2 mos. ago. Diagnosis fibrosarcoma	Mass over sacrum 10 cm. in diameter. Lymph node in both groins 2 cm. in diameter. Mass widely excised. Diagnosis fibrosarcoma. Radical treatment	2 mos. later hard, fixed lymph node in left groin. Radical dissection. Diagnosis metastatic fibrosarcoma rapidly growing in lymph node	X-ray treatment. Lung metastasis	3 yrs. 7 mos.	7 mos.	4 mos.	Dead
Palmer 47 W & S 23	48	M	Fibrosarcoma	Radical excision	Bent hand back in fall 1 1/2 yrs. ago, hurt again 1 yr. ago. 6 mos. later swelling, dislocation 1 wk.	Hard tumor 5 cm. in diameter over dorsum of forearm. Slight perosteal deformity by X-ray	When first seen. Amputation of forearm. Dissection of axilla. Diagnosis fibrosarcoma with metastasis to lymph node	Pulmonary metastasis	2 1/4 yrs.	1 yr.	1 yr.	Dead 1 yr. later at home
Pondville 11602	55	F	Fibrosarcoma	Radical excision	Lump right breast 1 1/2 yrs. ago. Biopsy diagnosis tumor. Radical mastectomy. Diagnosis fibrosarcoma. X-ray 2 mos. later showed no recurrence. Pain in legs 6 wks. ago, in arm 3 wks. ago.	X-ray showed metastasis to lungs, vertebra, ilium. Legs became paralyzed	At autopsy. Diagnosis fibrosarcoma, metastasis to lungs, pleura, liver, gall bladder, peritoneum, bone, lymph node		1 yr. 7 mos.	—	0	Dead
III-31-897 W & S 21	56	M	Fibrosarcoma	Radical excision	Pea-sized nodule medial to right nipple 6 mos. ago. Grew, excised 4 mos. later. Diagnosis fibrosarcoma	7 by 5 by 3 cm. red, non-tender tumor. Radical amputation right breast. Diagnosis fibrosarcoma with lymph node metastasis	At operation (when first seen)	A few nodules appeared in wound, unchanged for 2 yrs. Liver palpable for first time at last visit 4 mos. ago	6 1/2 yrs.	6 yrs.	6 yrs.	Living

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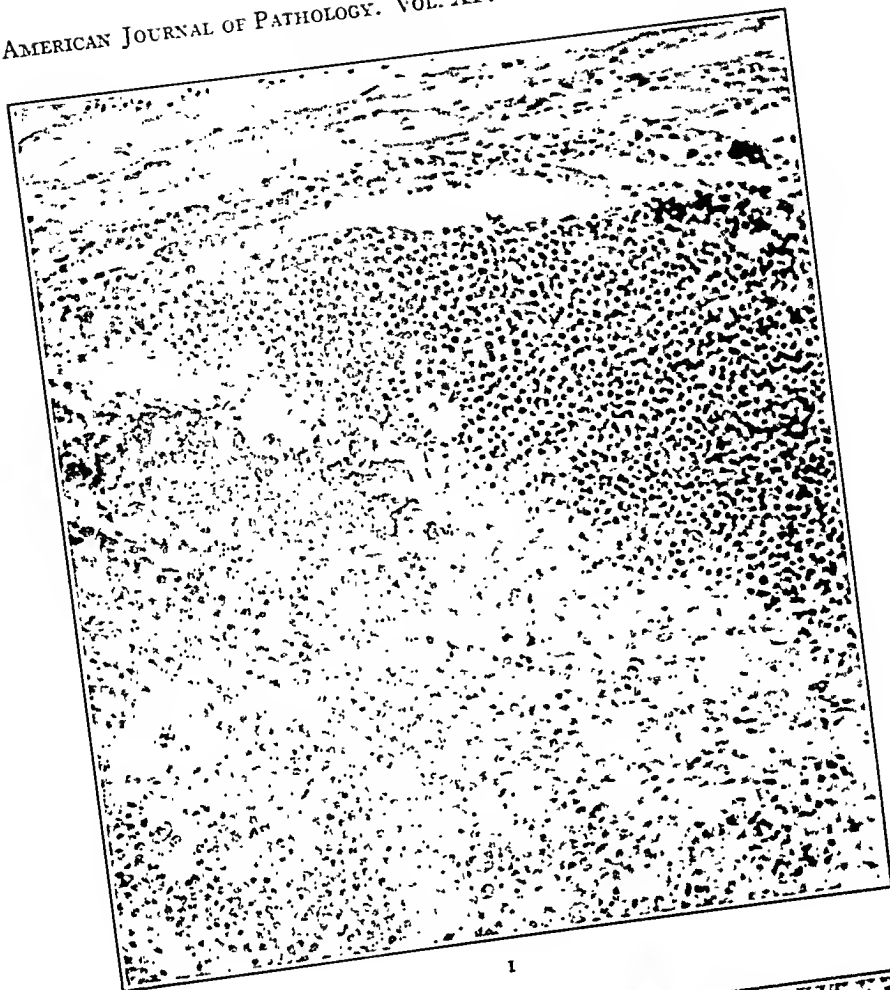
SUMMARY AND CONCLUSIONS

1. Lymph node metastasis occurs in 5 to 10 per cent of all hospital cases of sarcoma (exclusive of melanosarcoma, lymphosarcoma and clinically benign leiomyosarcoma of the uterus).
2. Seventeen cases of microscopically proved lymph node metastasis are presented.*
3. One patient is now living 6 years after removal of involved nodes.
4. Lymph node dissection with radical operation for removal of a sarcoma may improve the prognosis 5 to 10 per cent and may be valuable even after a lymphatic metastasis is clinically evident.

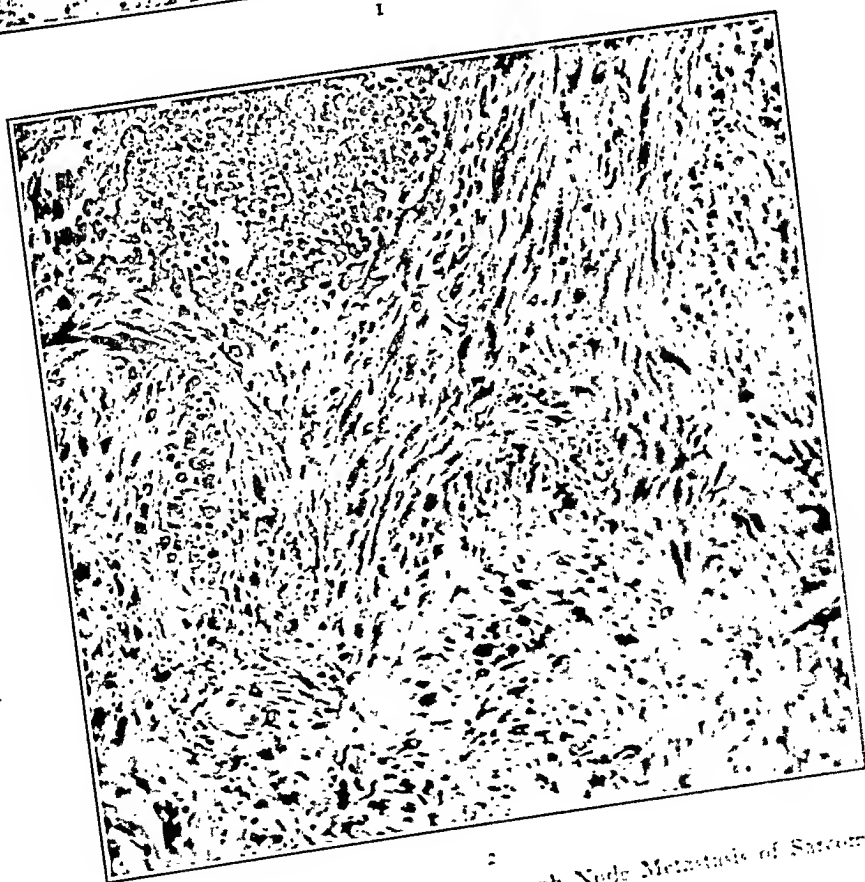
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* A few of these have been previously mentioned in another connection.



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2

Lymph Node Metastasis of Sarcoma

Warren and Meyer

DESCRIPTION OF PLATE

PLATE 139

- FIG. 1. Axillary lymph node showing metastatic fibrosarcoma. Primary focus subcutaneous in right abdominal wall. Phosphotungstic acid hematoxylin. $\times 300$.
- FIG. 2. Cervical lymph node showing metastatic fibrosarcoma. Primary focus in lower lip. Phosphotungstic acid hematoxylin. $\times 300$.

PRIMARY LIPOSARCOMA OF BONE *

REPORT OF A CASE

JAMES DUFFY, M.D., AND FRED W. STEWART, M.D.

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Primary liposarcoma of bone is sufficiently rare to justify the reporting of isolated instances of this disease. The existence of liposarcoma of bone was noted by Ewing¹ at the London Cancer Congress of 1928. One of the authors² reported in detail 3 cases from this hospital, 2 of which had formed the basis for Ewing's tentative recognition of the disease, the other case having appeared after the Congress report.

The tentative recognition of these tumors as liposarcomas was criticized by W. G. Barnard.³ He states that "case 2 appears to be a polymorphous-cell sarcoma in which some of the cells have taken up fat." The authors do not recognize the term "polymorphous-cell sarcoma" as other than purely descriptive since it implies nothing as to histogenesis, fundamental nature and presumptive clinical course. It has long been our effort to avoid such terms whenever possible. Barnard's criticism suggests a lack of familiarity with the alveolar lipogenic tumors of soft tissue. Fender⁴ reported a liposarcoma with certain alveolar tendencies although he makes no claim for its origin in bone but believes that it arose at least in close proximity to the fibula. An additional case was reported by L. Barnard⁵ and two others by Rehbock and Hauser.⁶

We have, therefore, 1 case of questionable bone origin and 6 where the origin in bone seems reasonably well established. The present case appears to be the 7th. The report of this last tumor has been deliberately delayed until the lapse of five years from first admission to the hospital in order that any peculiarities in the clinical course might be observed. It will be remembered that the original cases from this hospital ran clinical courses that seemed to place them in a special category apart from the usual sarcomas of bone.

* Received for publication June 7, 1938.

in 500 r doses through each portal. Reddening and superficial desquamation resulted and the recurrent mass showed considerable regression. It became fluctuant and some 300 cc. of bloody fluid were aspirated. The lung fields remained clear and no bone destruction could be demonstrated in the femoral stump. In February of 1934 tissue destruction was noted on the lateral aspect of the stump and persistent disease was palpable in the deeper tissues so additional radiation was given to two fields, anterior and posterior, totalling 1500 r per portal in 500 r doses. A biopsy in June 1934 showed no evidence of residual disease. By July 1934 the combination of heavy radiation and careless stump hygiene had resulted in considerable necrosis both anteriorly and posteriorly over the stump. The bone began to protrude and the infection spread. It was thought moreover that deep disease was present. Further radiation was impossible and a trial of Coley toxin was suggested. Toxin was given for 1 month, from Oct. 8, 1934 to Nov. 17, 1934, and from Nov. 30, 1934 to Dec. 22, 1934. This treatment was resumed in January and continued throughout the month. Constitutional reaction was moderate.

On Feb. 1, 1935 the femoral stump was disarticulated. The healing was incomplete and Reverdin grafts were necessary. Four months later the patient developed cough, with yellowish white sputum, occasionally blood-tinged. Radiographs of the lung fields showed for the first time evidence of metastasis. The metastatic deposit consisted of a single, bulky, sharply outlined round mass in the hilar area on the left. The mass measured 9 cm. in diameter. The mass was treated by radiation, through three portals, 500 r daily until each portal had received 1500 r. Toxins were continued, each course being conducted in such fashion that a reaction would be obtained every few days for a period of a month, after which a rest period of 2 to 3 months would intervene, to be followed by a continuation of the toxins. This treatment has been continued to date.

Following either the radiation or the toxins, or both, the lung metastasis regressed. It measured 9 cm. in diameter on Aug. 28, 1935. By Jan. 23, 1936 it measured 5 cm. in diameter. Six months later the size had decreased somewhat more and there was a very noticeable decrease in the density. In January 1938 no definite mass could be made out but the region showed considerable fibrosis

REPORT OF CASE

Clinical History: M.Q., male, aged 49 years, applied to the Memorial Hospital on March 28, 1933. His family and past history were irrelevant. He dated his present illness to a fall, sustained on Nov. 23, 1932. He stated that while carrying a load of 100 pounds he had slipped. He was admitted to another hospital where radiographs disclosed a fracture of the lower portion of the shaft of the left femur. The patient was treated for this fracture and on Jan. 7, 1933 was discharged with a supporting splint.

Although intermediate films are not available for study it would appear that healing was imperfect. Pain persisted and shortly before entering the Memorial Hospital the patient sustained a second fracture while lying in bed. Films taken the day following admission revealed a pathological fracture through the lower third of the left femur with extensive displacement. The bone tissue in the region of the fracture had a "rotten wood" appearance. There was a large soft tissue mass surrounding the site of fracture and at the periphery of the mass evidence of calcification. The differential diagnosis lay between a primary medullary tumor of bone, *i.e.*, plasma cell myeloma, and a metastatic carcinoma. No evidence of metastases appeared in films of the lungs. No other primary site of tumor was demonstrated.

It was decided to amputate the extremity. This may seem an unusual decision in view of the tentative diagnosis of myeloma but experience has shown that the bulky myeloma, through which fracture has occurred, does not do well with roentgen therapy. The tissue is usually reduced to a hemorrhagic cellular mass, often mainly blood clot, and the reaction essential to regression of tumor under X-ray proceeds very poorly under such circumstances. The fracture persists and isolated myelomatous areas remain throughout the hemorrhagic residue.

Amputation at a level 14 cm. below the uppermost point of the great trochanter was done on April 3, 1938. This level was well above the area of change demonstrated by radiographs. Convalescence was rapid and uneventful and within 3 months the patient was using an artificial limb.

Nine months after the amputation an enlargement of the stump was noted. The tissue was aspirated and a diagnosis of recurrent tumor was made. The stump recurrence was irradiated through three portals, anteriorly, posteriorly and laterally, giving 2000 r,

Staining for fat droplets revealed none in the spindle cells making up the bulk of the tumor. Only in the irregular islands of tumor in the medullary portion of the shaft above the main mass is it possible to trace the histogenesis of the lesion. In these areas there is a low grade chronic inflammatory reaction in the marrow fat tissue, characterized by diffuse and nodular lymphocytic infiltration, the presence of scattered large adult fat cells surrounded by many smaller young fat cells (Figs. 3, 4) with numerous fine, foamy droplets which take fat stains. In the adult fat cells the nuclei occupy the usual peripheral position. In the small vacuolated foam cells the nuclei are either peripherally or more centrally placed; some are small, rounded and deeply staining; others are larger, occupying from one-third to one-half the diameter of the cell. Occasionally the nuclei reach large dimensions and contain single large nucleoli as large as the entire nucleus of some of the young vacuolated fat cells. In the midst of these rounded or polyhedral fat cells are found fusiform cells which contain hyperchromatic ovoid nuclei. Such cells are obviously neoplastic and yet the cytoplasm of the fusiform cells appears vacuolated, thus resembling that of the fat cells. As the cells become more atypical vacuolization is lost, the cytoplasm tends to be rather acidophilic and the cell can no longer be identified as arising from fat.

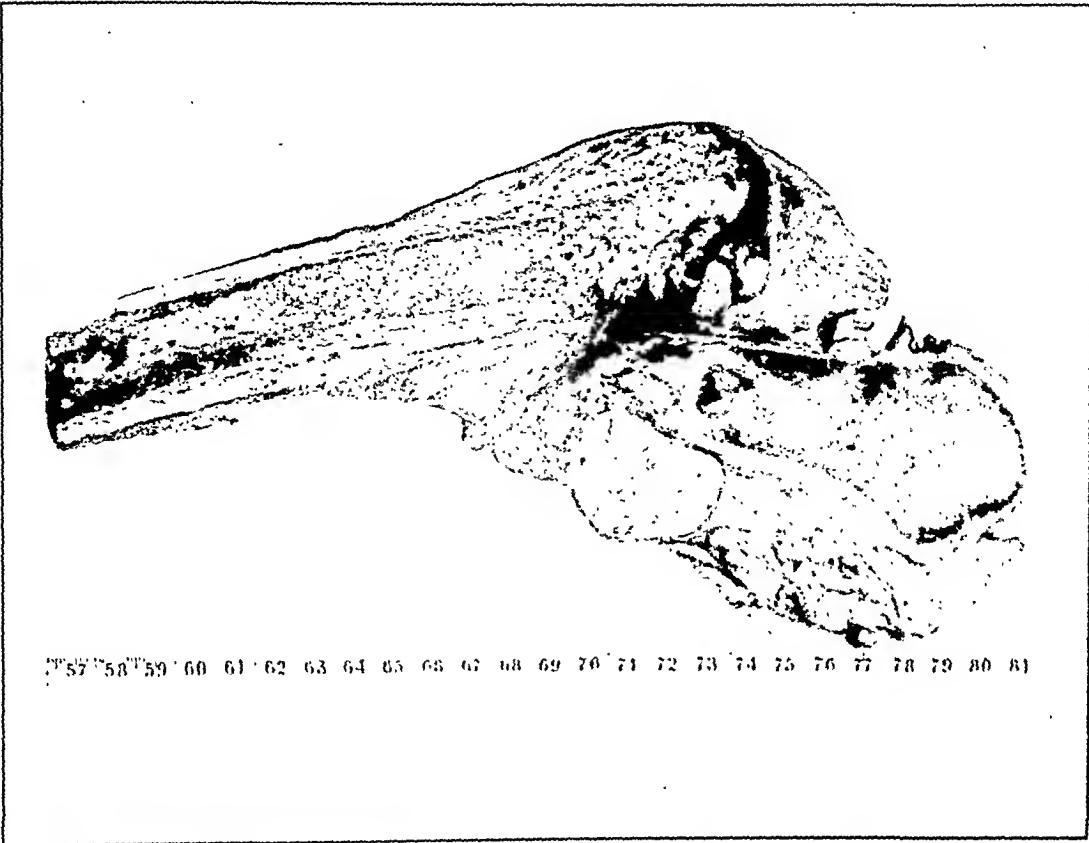
In our opinion the origin of this tumor is traceable to inflammatory changes in adult fat. The process is similar, for example, to the changes in fat which one occasionally sees in the kidney bed and which give rise to lesions that may be classed as true malignant tumors, or which when present in less obvious form may tax the effort of the pathologist to find a precise classification. We class the tumor here reported as a primary liposarcoma of bone. Adopting the proposed separation of liposarcomas into two types, adult liposarcoma and myxoliposarcoma of embryonal structure, as suggested by Ewing⁷ for the liposarcomas of soft parts, the tumor would be classed as an adult liposarcoma. The several peculiarities which serve to separate this tumor from the usual spindle cell medullary sarcoma of bone have been sufficiently emphasized in the clinical discussion. Since evidence has gradually accumulated over a period of years, which may indicate a relation between trauma, especially when repeated, and the development of liposarcoma of the adult type, the question might be

not incompatible with roentgen pneumonitis. The general condition of the patient remains excellent. He has gained much weight and weighs more without his leg than he did on admission with the extremity present.

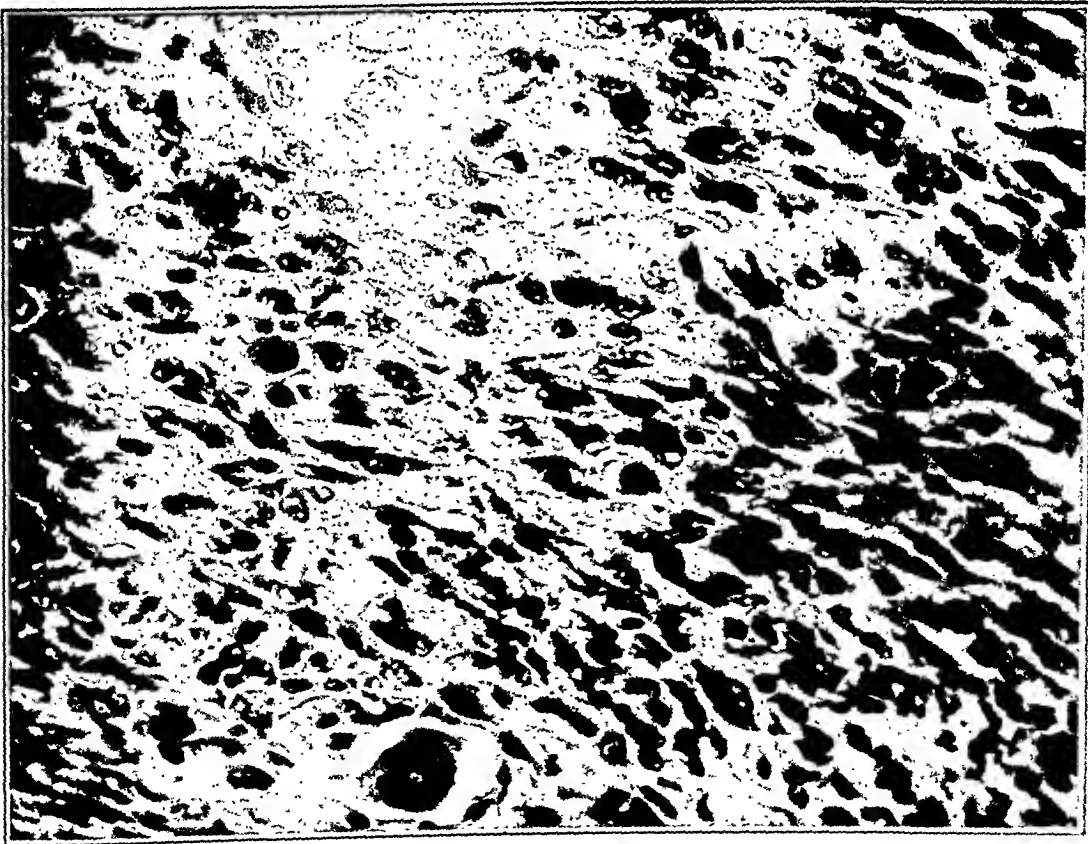
In other words, the entire clinical course of this tumor is unusual. Its natural history and response to treatment are not consistent with the usual medullary sarcoma of bone, nor can they be reconciled with a metastatic tumor. The apparent radiosensitivity coincides with previous observations on tumors of the liposarcoma group.

The peculiarity of the tumor became immediately evident from the gross examination. The tissue was soft, grayish yellow, lobulated, and revealed a coarsely fascicular structure, resembling a medullary fibrosarcoma of bone. The location, however, was distinctly unusual since such tumors tend strongly to occur at the extremities of the long bones. This tumor occupied the lower shaft, centering 11 cm. above the articular surface (Fig. 1). About 5 cm. of shaft were filled with rather sharply circumscribed tumor. A pathological fracture had occurred approximately in the center of the tumor-bearing area, the cortex was perforated and a sharply outlined, lobulated tumor had mushroomed out into the surrounding tissue, displacing muscles and an abundant deposit of extraperiosteal fat tissue. There were areas of bright yellow necrosis in the tumor. Extending up the shaft of the femur were irregular islands of glistening, opaque, grayish, mucoid-like tumor. A slight degree of callus production had occurred along the line of pathological fracture. There were foci of necrosis in the surrounding muscles. These were evidently the result of the trauma of fracture.

Microscopically the main tumor consists of interlacing spindle cells resembling those seen in medullary fibrosarcoma of bone (Fig. 2). They tend, however, to be a trifle more blunt in some areas than cells of the usual fibrosarcoma of bone. Their cytoplasm is more acidophilic and were the tumor one of soft tissues one would have strongly considered the possibility of muscle origin. The nuclei are largely centrally placed. The cells differ moderately in size and shape; mitoses are abundant and some few are atypical and multiple. Hyperchromatism of the large ovoid nuclei is marked.



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raised as to whether or not this tumor could have *followed* a fracture. It must be answered in the negative, since, although the initial films were not interpreted as showing tumor and the accident seems *bona fide*, a reexamination of these films in the light of subsequent events shows that tumor was present at the time the first fracture occurred.

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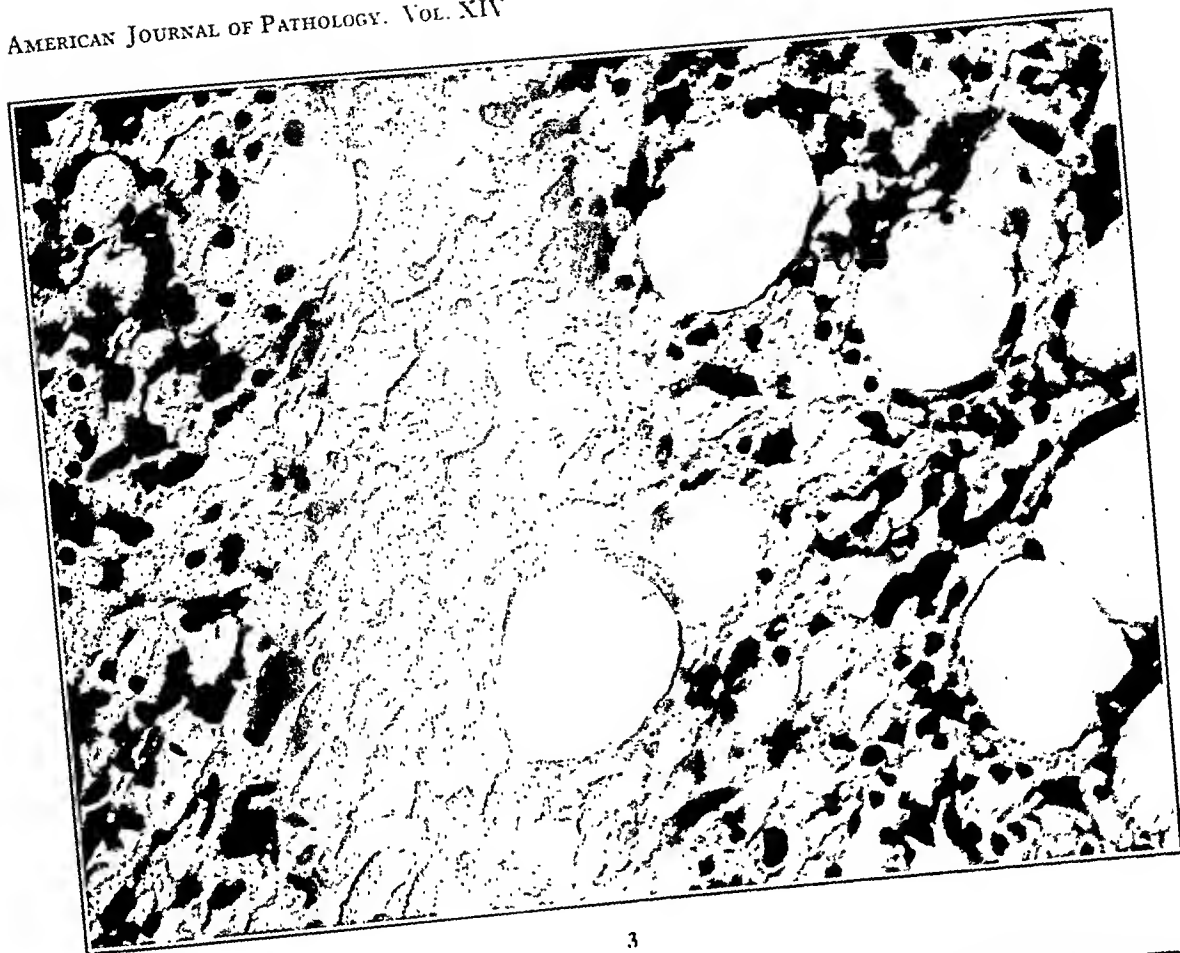
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DESCRIPTION OF PLATES

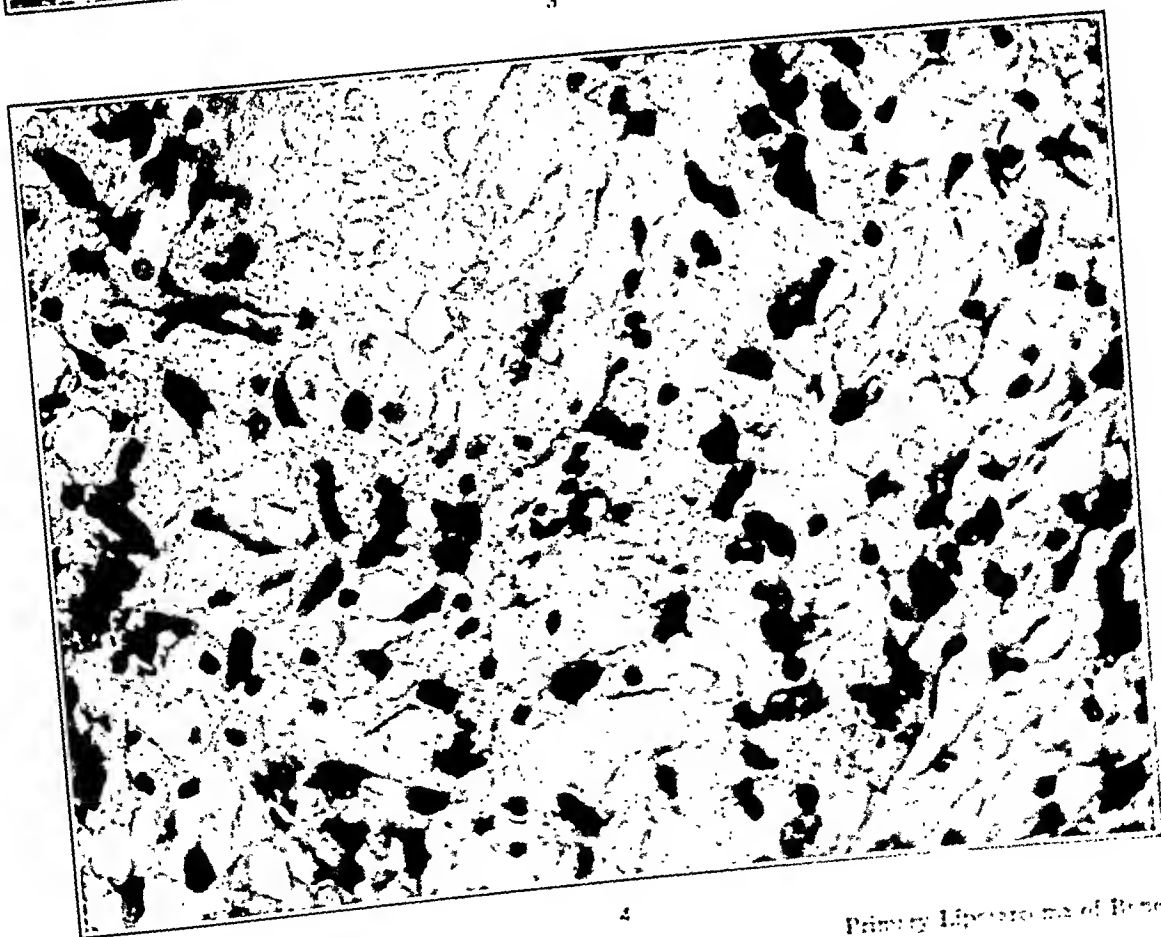
PLATE 140

FIG. 1. Hemisection of lower femur showing pathological fracture through the main portion of the tumor, well above the articular surface.

FIG. 2. Structure of the main tumor. Spindle and giant cell sarcoma.



3



4

Primary Liposarcoma of Bone

PLATE 141

FIG. 3. Adult fat cells and small vacuolated fat cells at the periphery of the main tumor. Origin of tumor cells from small young fat cells.

FIG. 4. A similar area apart from the main tumor. Fusiform vacuolated cells arising from fat.

SCIENTIFIC PROCEEDINGS OF THE
THIRTY-EIGHTH ANNUAL MEETING

OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

HELD AT ATLANTIC CITY,

NEW JERSEY

MAY 3RD AND 4TH, 1938

BUSINESS MEETING
OF
THE AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

Held in the Music Room, Hotel Chalfonte,
Atlantic City, N. J.

May 4th, 1938

PRESIDENT LONG PRESIDING

The Secretary presented the nomination of the Council for officers as follows:

<i>President</i>	EARL B. MCKINLEY
<i>Vice-President</i>	CARL V. WELLER
<i>Treasurer</i>	FRANK B. MALLORY
<i>Secretary</i>	HOWARD T. KARSNER
<i>Incoming Member of Council</i>	PAUL R. CANNON
<i>Assistant Treasurer</i>	FREDERIC PARKER, JR.
<i>Assistant Secretary</i>	HAROLD M. DIXON

Voted unanimously to elect those nominated.

Voted to elect the following new members:

George Packer Berry	Louis H. Koplik
Eugene Clark	Sidney C. Madden
Raymond O. Dart	Ernst E. M. Mathias
Walter B. Davis	Leo M. Meyer
Elbert DeCoursey	Alexander J. Nedzel
Paul Gross	Clarence I. Owen
Henry Horn	Isabella H. Perry
Walter W. Jetter	Silik H. Polayes
Homer D. Kesten	Edith L. Potter
John G. Kidd	Angelo M. Sala
Jack D. Kirschbaum	John A. Saxton, Jr.



AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

CHEMOTROPISM INDUCED BY STREPTOCOCCUS HAEMOLYTICUS IN LEUKOCYTES FROM NORMAL AND IMMUNE ANIMALS. Dale Rex Coman (by invitation), Morton McCutcheon and (by invitation) Paul T. DeCamp, Philadelphia, Pa.

Abstract. It is well known that phagocytosis of bacteria by polymorphonuclear leukocytes is greatly increased by the presence of specific antibodies (bacteriotropins) in the serum, but it is not known whether such antibodies play any part in directing the movement of leukocytes toward the bacteria, that is, in chemotropism. A favorable opportunity for obtaining information on this question occurred when we found a strain of hemolytic streptococci that only weakly and transiently attracted leukocytes of normal rabbits *in vitro*. If antibodies play any part in chemotaxis it seemed probable that addition of bacteriotropins would increase the attraction of leukocytes to this strain of organisms.

This hypothesis was tested as follows: Streptococci from a broth culture, after being washed, were transferred on a platinum loop to a glass slide, where they dried, forming a clump about 0.5 mm. in diameter. Polymorphonuclear leukocytes were obtained from the peritoneal exudate of a rabbit and were suspended in plasma from the same animal. A drop of this suspension was allowed to spread on the slide containing the bacteria. The preparation was sealed to prevent evaporation and was observed with the microscope at 37.5° C. Two sets of preparations were made; one with leukocytes and plasma from a rabbit immunized against the same strain of streptococci, and the other with cells and plasma from a normal animal.

The two preparations were observed alternately and the path of each leukocyte in the same microscopic field as the bacteria was recorded on paper by the use of a drawing ocular. The value of chemotropism was expressed as the average number of microns per minute of locomotion toward or away from the bacteria.

In both sets of preparations leukocytes were attracted by the streptococci for only a few minutes, and thereafter moved at random. The experiments showed no difference in chemotaxis between leukocytes from immunized and from normal rabbits.

Thus, chemotropism to hemolytic streptococci was not altered by using plasma and leukocytes from an immune animal, and no evidence was obtained that antibodies play a part in chemotropism of leukocytes.

Discussion

(Dr. Max B. Lurie, Philadelphia.) Is there any relation between the principle responsible for negative chemotropism and the old idea of aggressins?

(Dr. Coman.) I have no idea whether there is any similarity between the substances that cause negative chemotropism and those substances that were termed aggressins. That possibility has occurred to us, but we know little about the substances that cause negative chemotropism. I think it is too early to draw any conclusions.

Robert Schrek
Mark P. Schultz
Gertrude Silverman
Kenneth C. Smithburn
Gabriel Steiner

John Y. Sugg
Frederick Summerill
Robert Tennant
Robert M. Thomas
George Z. Williams

It was also voted to reinstate Dr. George T. Caldwell.

Voted to accept with regret the resignations of Drs. E. M. Eberts, M. Fernan-Nunez, N. MacL. Harris and Leila Jackson.

Voted to record with deep regret the deaths of Drs. J. E. Bates, C. A. Bentz, Louis Gross, O. V. Huffmann, R. H. Jaffé, E. H. Kettle, Oskar Klotz, G. F. Laidlaw, J. E. McWhorter, Sigmund Pollitzer, H. S. Thatcher, T. T. Walker and C. Y. White.

The Secretary announced that the next meeting of the Association will be held at the Medical College of Virginia, Richmond, Virginia, April 6 and 7, 1939.

The Secretary announced that the Symposium for next year will be on the subject of Hypertension, and that Dr. Harry Goldblatt of Cleveland, Ohio, had been selected as the Referee.

The Secretary called attention to certain changes in the Constitution and By-Laws proposed at the meeting last year.

Voted to adopt the following amendments and alterations to the Constitution and By-Laws:

Amend Article II of the *Constitution* to replace the words "Council of Seven, who shall" by the words "Council, which shall."

Replace Article 2 of the *By-Laws* with a new Article 2, to read: "The Council shall consist of seven Members elected by the Association, and the Secretary and Treasurer *ex officio*. The Members shall be elected for terms of seven years each, one Member to be elected annually, and shall not be eligible for immediate reelection."

Delete Article 3.

Change Article 4 to Article 3, and Article 5 to Article 4.

Replace Article 6 with new Article 5, to read:

"The annual dues shall be determined by the Council."

Change Article 7 to Article 6.

The scientific sessions proceeded as in the following program.

MULTIPLE VIRUS INFECTION OF INDIVIDUAL HOST CELLS. Jerome T. Syverton and George Packer Berry (by invitation), Rochester, N. Y.

Abstract. It has been known for many years that an individual host can experience several virus infections at one and the same time. The findings reported herewith indicate for the first time that several viruses may manifest their characteristic activities simultaneously within a single host cell. This coexistent infection has been demonstrated for the normal cells of the rabbit's cornea, as well as for cells of the virus-induced rabbit papilloma and of the squamous cell carcinoma which frequently follows the benign wart.

The viruses employed were the following: herpes virus, B virus, virus III, vaccine virus, *Virus myxomatosum* and Shope's papilloma virus. They were used in such combination that one of the component viruses in the mixture produced an inclusion body in the nucleus, the other virus an inclusion body in the cytoplasm. All of the experiments were made on rabbits, domestic or cottontail. In all, 92 animals were used. The conclusions are derived from the histopathological findings, the recovery of all of the viruses by animal passage, and the identification of each virus by suitable immunological procedures.

In addition to emphasizing the fact that tumor cells can be infected, or superinfected, with extraneous viruses, the findings on coexistent infection of individual host cells throw new light on the host-parasite relationship in virus infections. This may be significant in connection with the so-called "sparing effect" ("interference phenomenon") of one virus upon another (Thung, Salaman, Kunkel, Hoskins, Dalldorf, Findlay, and others).

Discussion

(Dr. Berry.) We have been interested in multiple virus infections of individual cells in relation to the so-called "interference phenomenon," the "sparing effect" of one virus upon another. That one virus strain may influence another has been known to workers with plant viruses for a number of years. Recently, Hoskins, Dalldorf and Findlay have presented evidence for the same sort of thing with animal viruses. How do our findings fit into the picture? There may not be any clash. After several viruses have been applied simultaneously to a rabbit's cornea, the vast majority of the cells parasitized show evidence of infection by but 1 virus — only in occasional cells do several viruses manifest their activities simultaneously. On the other hand, almost all the cells of the virus papilloma of Shope can be readily superinfected with extraneous viruses. Thus, although certain viruses may "interfere" with certain other viruses, this phenomenon obviously is not universal.

(Dr. Peyton Rous, New York City.) Do not these experiments bring up the possibility that the behavior and morphology of tumors may be influenced by extraneous virus infection? Many transplanted tumors can carry contaminating viruses with them. It is conceivable that spontaneous tumors may undergo intercurrent infection with viruses that would influence their growth and morphology.

(Dr. Thomas Francis, New York City.) In relation to Dr. Rous's comment, the observation of Findlay seems rather important because he found in mouse tumors he could carry the yellow fever virus indefinitely without influencing the growth of the tumor.

(Dr. Calvin G. Page, Boston.) Thirty years ago, in 1908, there was an outbreak of foot-and-mouth disease near Detroit. The origin of the outbreak was

SEROLOGICAL STUDIES OF CHILDREN BEFORE AND AFTER VACCINATION WITH THE SCARLET FEVER STREPTOCOCCUS TOXIN. Max M. Strumia, Bryn Mawr, Pa.

Abstract. Fifty children between the ages of 5 and 15 years and 5 adults between the ages of 18 and 21 years were vaccinated with commercial scarlet fever streptococcus toxin and studied serologically before and after the immunization. All showed a positive Dick test before vaccination. The vaccination consisted of 5 weekly doses containing respectively 500, 2000, 8000, 25,000 and 80,000 to 100,000 skin test doses, or a total average of 125,500 S. T. D. Agglutination tests against 3 strains of scarlatinal streptococci and antifibrinolysin determinations against 2 strains of scarlatinal streptococci were carried out on all individuals before and after vaccination. In addition, antistreptolysin determinations were done before and after vaccination on 37 of the patients. Whereas the Dick test became negative in all of the patients after vaccination, the other serological determinations showed little or no change.

The following control series was also studied: 8 normal adults (over a period of time similar to the vaccinated group); 20 children with a negative Dick test; and 8 cases of streptococcic infection, including 6 cases of scarlet fever.

THE ASCORBIC ACID CONTENT OF DERMAL LESIONS INDUCED BY DIPHTHERIA TOXIN. PRELIMINARY REPORT. Calvin C. Torrance, Albany, N. Y.

Abstract. Studies of the relation of vitamin C to generalized diphtheria intoxication have been extended to the dermal reactions induced by the injection of this toxin into the skin of 3 representative species of animals. The amount of ascorbic acid found in the areas of inflamed skin at various intervals of time after the injection of the toxin was compared with that of the uninjected skin from the opposite side of the same animal and was found to be relatively diminished during the acute phase of the reaction in guinea pigs and rabbits. As the lesions healed, the amount of vitamin C increased over that found in the normal skin of these animals. When comparable reactions were induced in rats by injecting 5000 times the amount of toxin used in the more susceptible rodents, the marked increase in the vitamin which was observed at 48 hours continued to the termination of the experiment.

Discussion

(Dr. Esmond R. Long, Philadelphia.) Will Dr. Torrance tell us briefly what is the principle of the method by which the ascorbic acid is determined?

(Dr. Torrance.) The method is essentially that of Bessey and King, which was designed to determine ascorbic acid in the tissues, but we have found it impossible to use this method, as it was originally described, because the skin cannot be ground in a mortar. The skin is minced with scissors and placed with acetic acid in evacuated tubes and heated in the water bath for 20 minutes. The tubes are then opened and the contents transferred to a centrifuge tube and the supernatant fluid removed. The piece of skin, softened by heating and acid, can then be washed 3 times with trichloroacetic acid. The pooled washings and the fluid in which the skin was heated are titrated with 2, 6-dichlorophenol-indophenol.

Discussion

(Dr. Harry S. N. Greene, Princeton.) I should like to ask if the papillomas occur on any other mucous surface.

(Dr. Jerome T. Syverton, Rochester, N. Y.) This is the second virus papilloma of rabbits to be described. Have you observed any instances of this new tumor becoming malignant? Does it undergo successive changes over a period of time similar to those described by Rous and his associates with the Shope papilloma? What is the longest period of time that you have had any of these tumors under observation?

(Dr. Peyton Rous, New York City.) Were there inclusion bodies in the cells of this papilloma?

(Dr. Henry W. Scherp, Rochester.) I was very much interested in the statement that rabbits which had been tarred had a higher incidence of papillomas, and I wonder if Dr. Parsons would care to say anything about the possible etiological significance of this fact.

(Dr. Parsons.) In answer to Dr. Greene, we have attempted to infect every available mucous membrane in the rabbit, and have been able to induce papillomas only on the oral mucous membrane. Most of our inoculations have been on the under surface of the tongue. In every animal in which the lesions were found they were on the under surface of the tongue, and in a few lesions were found also on the lips, the floor of the mouth, or the top of the tongue. We have attempted to produce the lesions on the oral mucous membranes of dogs, cats, rats and guinea pigs, and in none of them have we been able to obtain lesions, nor can we produce the lesions on the skin of these animals, or on the skin of rabbits.

In answer to Dr. Syverton, there were no changes of a malignant nature in these tumors. We have had some of them under observation for more than a year now. We have attempted to induce malignant changes and have failed to do so.

Dr. Rous asked about inclusion bodies. In about 1 per cent of the papillomas inclusion bodies were found. I did not describe them because they occur so infrequently. Whether they have any relation to this virus disease, or whether they are from another virus, we have no idea.

In answer to Dr. Scherp's question, I think all one can do is to theorize on that. It seems to me that we have shown beyond much doubt that the papillomas are caused by a filterable agent. I see no reason to suppose that tar contains that filterable agent. What I think happens is that these animals get tar in their mouths as a result of licking the tarred areas of their skin. The oral mucous membrane is irritated in the same way that the skin of rabbits is in experiments in which tarring of the skin is used as a means of localizing the Shope papilloma virus from the blood stream. I think the irritation of the mucous membrane allows the virus to gain entrance into the epithelial cells.

(Dr. Syverton.) May I ask if the inclusion bodies found in the 1 per cent of papillomas fulfill the requirements for Cowdry's type A or type B intranuclear inclusion bodies?

(Dr. Parsons.) They are type A acidophilic intranuclear inclusion bodies, and have been seen by Dr. Rivers and Dr. Rous.

finally traced to vaccine virus imported from a foreign country several years previously. This virus had been used for experimental purposes only, but a few of the calves inoculated with the virus were not destroyed, as they should have been. This incident is an interesting example of 2 kinds of virus existing together for a considerable period of time (see *U. S. Dept. Agric. Techn. Bull.*, No. 76, June, 1928, 1 and 2. Report of Foot-and-Mouth Disease Commission).

(Dr. Berry.) In connection with the remarks by Dr. Rous, I may say that we have noted that the cells of the virus papilloma of Shope may be superinfected with extraneous viruses very easily. Indeed, the papilloma cells are much more readily infected than those of the normal skin. This may be demonstrated either by local or intravenous inoculation of the extraneous viruses. Since the papilloma cells are young and growing vigorously, this finding is what one would expect. As has been pointed out, it has been known for many years that the actively growing cells of transplantable, but non-filterable, tumors can carry viruses.

(Question from audience.) May I ask how this work was carried on — with viruses injected simultaneously, or was it possible when a virus had established itself in a particular tissue to keep it in that particular tissue?

(Dr. E. L. Benjamin, Evanston.) What staining technic was used?

(Dr. Syverton.) The histological preparations from which the colored lantern slides (Kodachrome) were made were stained by Giemsa's method. We also stained sections with eosin-methylene blue and with phloxine-methylene blue.

In reply to the question whether we injected the different viruses simultaneously or at different times, we did both. In most instances, however, the 2 or 3 viruses were introduced simultaneously. In the case of the papilloma virus it was obviously necessary to introduce this agent several weeks in advance in order to provide actively growing papillomas.

ORAL PAPILLOMATOSIS OF DOMESTIC RABBITS: A VIRUS-INDUCED DISEASE.

Robert J. Parsons and John G. Kidd (by invitation), New York City.

Abstract. Small discrete papillomas are frequent on the oral mucous membranes of domestic rabbits, usually appearing on the under surface of the tongue. Their incidence in the 732 domestic rabbits examined varied widely in different groups, being 9.6 per cent in normal rabbits and 42.8 per cent in animals whose ears had been tarred. None were present in any of 312 wild cottontails.

The growths are benign, consisting of a proliferating layer of abnormal, stratified squamous epithelium supported on delicate connective tissue papillae. Berkefeld V or N filtrates of Tyrode extracts of the papillomas will cause the disease on inoculation into the traumatized oral mucous membranes of both domestic and cottontail rabbits, and it can be propagated serially with filtrates in both species. The filterable agent can be preserved in glycerol for a year at least, and it remains active when frozen and dried. Its activity is reduced slightly by a temperature of 65°C. for 30 minutes, but 70°C. for 30 minutes largely inactivates it, and 75°C. does so completely. A few domestic rabbits have been found immune on inoculation with the virus, as the result probably of previous infection, since individuals bearing the growths are usually immune. Such animals, however, are fully susceptible to the skin papilloma virus (Shope), and animals immune to the skin papilloma virus are susceptible to the oral papilloma virus.

The induced papillomas usually retrogress, but some have persisted for more than a year. No tendency to malignant change has been noted.

affected. In such foci the damage to neurones appears to be secondary to the inflammatory reaction.

Two types of lesions are clearly distinguishable, the inflammatory and the degenerative, although there may be a certain degree of overlap. Several examples of each type are described. The inflammatory response is considered to be the primary reaction to the disease agent after peripheral inoculation.

Analysis of the distribution of lesions in early symptomless cases shows that the virus passes directly from the blood into the brain tissue. The presence of lesions in the cerebral cortex with intact subcortical centers is held to exclude nerve transmission. Under certain conditions, however, the virus may "travel along nerve paths."

Discussion

(Dr. Jerome T. Syverton, Rochester, N. Y.) Dr. King, have you any evidence, other than experimental, that the mosquito or the chicken can be implicated in the transmission of equine encephalomyelitis? We have been interested in the epidemiology of this disease and, as you know, have shown that the wood tick, *Dermacentor andersoni*, can transmit the infection in the laboratory.

A second question concerns your results following peripheral and intracerebral inoculation. To make my question clear, most of the strains of the Eastern type of equine encephalomyelitis are highly virulent. Intracerebral inoculation of such strains usually kills guinea pigs in 24 to 48 hours, while with peripheral inoculation the period is prolonged for at least an additional 1 or 2 days. A prolongation of the disease results in proliferative changes with polymorphonuclear invasion. Did the marked differences that you describe in respect to the two routes of inoculation follow a short incubation period, or was there a considerable prolongation of the clinical disease following peripheral inoculation which might account for the observed differences in the histopathological reactions?

(Dr. Albert E. Casey, University, Va.) In connection with the St. Louis encephalitis, where we had a second epidemic last summer, the epidemiology seems to be identical with that which Dr. King noted in encephalitis in horses, and the lesions were quite similar. The disease seems to be located around waste lands and about the streams in the vicinity.

(Dr. King.) In answer to Dr. Syverton's first question, so far as I know there is no evidence other than experimental that mosquitoes and fowl are involved in the transmission of the disease.

In regard to the relation between peripheral and intracerebral inoculation, the intracerebral inoculation, with the strain of virus and dosages used, results in death in 3 to 5 days; with very massive doses the animal may die in 48 to 72 hours. However, the animals which were used for illustrations survived 4 to 5 days, and thus were comparable with those inoculated peripherally. I do not feel that the difference in pathology can be explained merely on the time factor. Instead, there seem to be two fundamental pathological processes involved which are partly overlapping.

In reference to the St. Louis encephalitis, I can say nothing about the epidemiology of the disease, but I think it is interesting that Webster in his study of this disease in mice pointed out that the first lesions existed in the interstitial tissue and that nerve cells were involved only secondarily. This is similar to my own findings with equine encephalomyelitis.

PROPAGATION OF THE VIRUS OF HUMAN INFLUENZA IN THE GUINEA PIG FETUS.*

O. C. Woolpert, F. W. Gallagher and Leona Davis (by invitation), and N. Paul Hudson, Columbus, Ohio.

Abstract. Using a technic previously described, the P. R. 8 strain of influenza virus (human) was inoculated intracerebrally into fetal guinea pigs approximately 35 to 40 days of age. The virus was found to multiply and disseminate widely in fetal tissues. Titters of 10,000 or more, as determined by the mouse test, were commonly attained in fetal lungs. Liver and placenta also contained good quantities of virus. A 48 hour incubation period was found to be favorable. Two series of passages through fetal guinea pigs have been accomplished — one of 10 transfers, the other of 16, the latter being still in progress. The identity of the virus was maintained during passage, as demonstrated by suitable cross protection and cross neutralization tests in mice, using the original strain as a basis of reference. It was found that the fetal guinea pig could be infected with amounts of virus that produced little if any gross response in test mice, and that starting with such material one could obtain virus of good titer after 1 or 2 passages in fetuses. We conclude that the fetal guinea pig is useful as an experimental animal for influenza studies and for the production of bacteriologically sterile influenza virus in quantity.

Discussion

(Dr. James W. Jobling, New York City.) I should like to ask what time elapsed between the inoculation of the fetus with virus and its removal to be tested.

(Question from audience.) I should like to ask whether any evidence was obtained that in the mother there was any increase or presence of viral antibody. I think it is interesting that Dr. Hudson showed the virus may be recovered from the blood. Did you find the virus was concentrated more in the red blood cells, or did you use whole blood?

(Dr. Hudson.) The usual incubation period was 2 days, although we have used between 2 and 6 days in the fetus.

We have not studied the antibody in the mother in these cases. We have with the vaccinia virus, and find there is a low titer of the antibodies produced in the mother, that is, after the fetus has been inoculated with the virus. There is an interesting relation between the mother and the fetus and we have worked it out chiefly in mice. There seems to be a possibility of infecting the mother by inoculation of the mouse fetus.

Replying to the question of influenza virus in the blood, we used whole blood, and have not attempted to identify the virus in any element of the blood.

THE PATHOLOGY OF EASTERN EQUINE ENCEPHALOMYELITIS IN THE GUINEA PIG. L. S. King (by invitation), Princeton, N. J.

Abstract. Special attention was paid to the pathological findings of the central nervous system in the early stages of the disease before symptoms were apparent. After subcutaneous inoculation of the virus the typical lesion is an isolated, fairly well circumscribed focus of polymorphonuclear leukocytes found principally in the cerebral cortex, although any portion of the brain may be

* Aided by a grant from Eli Lilly and Company.

To the serological and animal inoculation tests one may add cultural studies. There are several kinds of culture media (liquid and semisolid) on which one can readily culture the *Leptospira icterohaemorrhagiae* from the blood of experimental animals and from individuals suffering from acute leptospirochetosis.

About 60 representative samples of blood serum were obtained out of about 300 cases from recent outbreaks of infectious jaundice in Michigan, about 150 cases in Windber, Pennsylvania for agglutination tests, and 15 samples of urine and whole blood for animal inoculation tests. Of these, so far only 3 samples have given prompt and clear-cut positive agglutination reactions with our Type I *Leptospira icterohaemorrhagiae* (titer over 1:40,000 reaction completed within 2 hours). Animal inoculation tests were all negative. Further studies on these samples are in progress.

During the last 2 years a large percentage of our positive diagnoses in human beings and in dogs has been based almost exclusively on agglutination tests because the cases are referred to us too late for animal inoculation tests.

The human serum which has been giving strongly positive reactions (in the writer's hands) during the last 2 years came from the following States: New York, Massachusetts, Pennsylvania, Maryland, Ohio, Michigan, Missouri, Kansas, Iowa, Tennessee, Florida, Texas, and also from Canada.

Discussion

(Dr. Joseph Kasper, Detroit.) Our attention was first called to spirochetel jaundice a few months ago when a large number of children were absent from school on account of an outbreak of jaundice. In one locality 12 children were affected at about the same time. While studying the problem the Department of Health encountered 1 case which was diagnosed as infectious jaundice. A 3 year old boy — the only child in the family — became ill and showed icteric discoloration of the skin about 3 days after his dog was returned from a veterinary hospital where it was treated for jaundice. At first the child did not seem very ill and examinations of his blood did not reveal the presence of *Leptospira*. Two weeks following the onset of his illness he became critically ill, the jaundice increased in intensity and he was removed to a hospital. At that time dark-field examination of the blood resulted in the finding of 1 organism that had the appearance of *Leptospira icterohaemorrhagiae*. Upon this evidence the diagnosis of infectious jaundice was made. Several hours after being admitted to the hospital the child died. Autopsy revealed multiple hemorrhages in the lungs and peritoneum, and marked degeneration of the liver. Microscopic sections showed diffuse necrosis of the liver cells. Sections of the liver and kidneys stained by Dieterle's method showed the presence of *Leptospira*. In the meantime the serum of the patient was sent to the National Institute of Health to be tested for agglutinins against *Leptospira*. This test revealed atypical agglutination in a titer of 1:30,000. The blood of the dog was also examined for these agglutinins and the reaction was more specific in equally high titer. Blood from 2 other dogs that had recovered from jaundice likewise contained agglutinins against *Leptospira icterohaemorrhagiae*. Following this experience a large number of wild rats were examined and in only 2 of these animals have we been able to demonstrate *Leptospira* in the tissues. One of these strains has now been carried through the 5th passage in animals and produces jaundice invariably in the guinea pig. It is believed by some that a specially bred strain of guinea pigs is necessary for the diagnostic inoculation, but we have found that this is not necessary. A white guinea pig serves better for the earlier detection

THE PREVALENCE OF INFECTIOUS JAUNDICE IN THE UNITED STATES AS DETERMINED BY AGGLUTINATION AND ANIMAL INOCULATION TESTS. Ardzoony Packchanian (by invitation), Washington, D. C.

Abstract. Outbreaks of infectious jaundice have been reported from practically every state in the Union. As a rule these cases are readily recognizable clinically (yellow color, nausea, vomiting, fever, bile-stained urine, albuminuria, muscular and epigastric pain, and so on). The cases are usually mild and the mortality is very low. The patient suffers for about 1 to 3 weeks and then recovers with or without medical care. The clinicians for convenience have diagnosed these cases as catarrhal jaundice; only a few cases have been suspected and diagnosed as Weil's disease. What percentage of these infectious jaundice cases is due to leptospirochetosis and what percentage is due to filterable viruses, or to other microorganisms, has been and still is an unanswered question.

The diagnosis of Weil's disease or icterohemorrhagic spirochetosis in the past was considered difficult because of the lack of proper laboratory methods of diagnosis. However, during the last few years we have had two new laboratory procedures for diagnosing this disease and for further experimental studies. One of these methods is the agglutination-lysis test introduced by Schüffner, and the other is the inoculation of animals newly found to be susceptible — certain species of American deer mice introduced by the writer as test animals.

The agglutination test which is used by the writer at the National Institute of Health is essentially Schüffner's method with certain so-called modifications. It is similar to typhoid agglutination tests. We use both living and dead antigen. For routine diagnostic work we prefer formalized antigen of *Leptospira icterohaemorrhagiae*.

We have at the present time at least 4 serological types of *Leptospira icterohaemorrhagiae*, the 4th type or group representing several different antigenic strains. When serum is received for diagnosis it is first tested with Type I, in duplicate, and in various dilutions for the sake of convenience, up to 1:30,000. When the result is strongly positive within 2 hours, it is reported so, and the remaining serum is saved for further studies. When the serum fails to agglutinate with Type I, other types are tried. Or it is reported "it failed to agglutinate with our Type I, *Leptospira icterohaemorrhagiae*."

Each serological test is done by monovalent antigen. The polyvalent antigen at the present time is not used for routine serological studies.

The writer has found several species of American rodents susceptible to *Leptospira icterohaemorrhagiae* and has selected two species of American deer mice, *i. e.* *Peromyscus eremicus*, and *Peromyscus maniculatus* (albino and hairless) for the following reasons: (a) for uniformity and for the correlation of results of different workers; (b) because the breeding stock of these animals in captivity is already established; and (c) because the jaundice is relatively easily noticed in albino and hairless mice (*P. maniculatus*).

Albino American deer mice, *Peromyscus gambelis*, young *Peromyscus eremicus* and young albino guinea pigs are inoculated about the same time with the sample of urine or defibrinated blood from a case of suspected Weil's disease.

When an inoculated guinea pig fails to die and *Leptospira* are not demonstrable in its blood at the end of the 4th week, its heart's blood is removed and tested for the presence of agglutinins and lysins, and the organs are sent to the Division of Pathology for microscopic pathological findings and for search for *Leptospira* in the tissues.

(Dr. Packchanian.) During my recent field studies in Michigan I discussed and demonstrated to Dr. Kasper how easily one may confuse blood filaments and fibers with *Leptospira*, also the fact that any color of guinea pigs could be used for the purpose of diagnosis. However, preference should be given to young albino guinea pigs for obvious reasons. With some strains of *Leptospira icterohaemorrhagiae* even very young guinea pigs may fail to contract demonstrable infection. In other strains jaundice may be absent altogether, yet there will be leptospirosis. Spontaneous laboratory infections in guinea pigs with *Leptospira* are also known. Such guinea pigs, if they do recover, are obviously not suitable for inoculation tests.

The fact that the child gave a slow partial agglutination with one of our strains of *Leptospira icterohaemorrhagiae* does not necessarily prove that the death was caused by leptospirosis (Weil's disease) rather than by another disease.

After performing the agglutination tests with this child's blood, we wrote to the Detroit Board of Health, suggesting the possibility of dogs acquiring spirochetosis from rats and carrying it to humans, and requested samples of blood for tests. Three samples of dog blood received at that time all gave strongly positive agglutination reactions with our human strain *Leptospira icterohaemorrhagiae*, the titer being over 1:30,000.

The most clear-cut case of Weil's disease in Michigan during the past year was that of a male about 32 years of age (Tushman), whose serum gave a definite agglutination reaction during the 2nd and 3d weeks after onset of illness. The agglutination titer was over 1:30,000, and the reaction was prompt and completed within 2 hours.

In reply to Dr. Syverton's and Dr. Tannenberg's questions, we are using, as a rule, microscopic agglutination tests originally introduced by Dr. Schüffner. We use both living and formalized antigen. For routine work we prefer dead antigen (formalized). The percentage of positive results is very high with agglutination tests, and extremely low with animal inoculation and cultural tests. While *Leptospira* are easy to demonstrate at certain periods in the blood of experimental animals, it is almost impossible to do so in human cases. A few positive results reported in the literature may be *Leptospira* or may be the confusion of blood filaments and fibers with *Leptospira*.

Dr. Hudson's question is a very important one. At the present time we have at the National Institute of Health, U. S. Public Health Service, about 40 strains of *Leptospira icterohaemorrhagiae*. These strains came from various parts of the United States and from Brazil, England, Germany and Hungary. In our collection we have strains derived directly from humans, from rats and dogs, and from water. We have grouped these strains, at the present time, for convenience, into 4 serological types or groups, the 4th of these groups being miscellaneous strains. In all probability there is an overlapping, and possibly there are other serological strains as yet undiscovered. This line of study is in progress.

In reply to Dr. Berry's question, I would like to state that "*Leptospira canicola*" is only a strain of *Leptospira icterohaemorrhagiae* that we have placed in our Group III. We have examined serum from dogs which suffered from leptospirosis with our human strain *Leptospira icterohaemorrhagiae* (Group I) which gave a very strong agglutination titer — 1:40,000. Man, dogs and rats can be infected with different serological strains of *Leptospira icterohaemorrhagiae* and just because the strain came from the dog, naming it "*Leptospira canicola*" as a new species does not seem warranted.

of icterus, but we have used colored animals with good results. Whenever the test animal shows the first definite signs of illness, it is justifiable to obtain blood from the heart. Large numbers of the organisms may thus be found on direct examination of the blood.

(Dr. Joseph Tannenbergh, Albany.) I should like to ask whether the spirochetes are killed by the agglutination.

(Dr. N. Paul Hudson, Columbus.) Did you examine strains from different parts of the country and find that they were antigenically similar by complement fixation, agglutination, or cross-immunity tests?

(Dr. George P. Berry, Rochester, N. Y.) I wish to ask Dr. Packchianian to elaborate on some of the statements which he has just made. The points involved concern our findings with experimental Weil's disease and our experiences in the field. Possibly these points can be brought out best in a series of questions. Dr. Packchianian, have you recovered *Leptospira canicola* from a human case, or a dog to which a human case has been traced? Was the boy, D. S., who died in Detroit on Feb. 14, 1938, suffering from a canicola infection? Did you actually recover *Leptospira* from this boy? Did you conclusively demonstrate the presence of specific antibodies in his serum? From how many, if any, of the "300 cases in Michigan" have you recovered leptospira or demonstrated agglutinins? Did you recover leptospira from any of the recent cases of jaundice in Detroit? In how many instances have you confirmed a positive agglutination test by recovering the etiological agent *Leptospira icterohaemorrhagiae*?

Among other epidemiological studies, we have recovered virulent strains of *Leptospira icterohaemorrhagiae* from 16 per cent of 42 wild rats trapped in Detroit during September, 1937. Furthermore, at that same time we recovered a virulent strain of this organism from a human case of Weil's disease in Detroit. As far as we know this is the first instance of this disease to have been proved in this city.

(Dr. J. Furth, New York City.) In a strain of common laboratory mice we occasionally found spirochetes identical morphologically with the spirochetes shown by Dr. Packchianian. These spirochetes could be passed to X-rayed mice very readily, but not to non-X-rayed mice. Moreover, mice X-rayed 24 hours after the inoculation came down with the disease. The blood infection was discovered accidentally by studying the blood of these animals for leukemia. It seems as if irradiation could be used to detect carriers and to render resistant mice susceptible to the disease.

(Dr. Jerome T. Syverton, Rochester, N. Y.) We have studied over 800 guinea pigs infected with 34 strains of leptospira, and one of the interesting points from the diagnostic standpoint with which we have been confronted is the difficulty of making an early diagnosis and isolating the organism. We have found that jaundice is not characteristically produced by strains of low virulence. Only 1 per cent of the guinea pigs inoculated with such strains show jaundice. Fever occurs in 80 per cent. The extent of the internal damage parallels the jaundice. The hemorrhages are confined to the lungs in about 80 per cent and are generalized in only a few animals. Obviously, it is often impossible to make a diagnosis simply by looking at a guinea pig. We often have guinea pigs with neither fever nor jaundice and yet on the 10th day we can isolate the organism by further passage. The second point concerns the agglutination method used by Dr. Packchianian. Was it the Schüffner method? Were formalized suspensions of organisms, living organisms, or what, employed?

In spectrums, growths are concentrated in bands, their number, type, variation and density depending on the organism. Concentration in bands makes growths frequently observable where they would be missed or absent in broth or streaks on slants, because of growth sparsity or inability to establish a favorable oxygen-medium relation for growth (unlike certain fungi, many bacteria seem unable to invade solid medium to a favorable oxygen tension for growth (*J. Lab. & Clin. Med.*, in press)).

Depth of growth increases for certain organisms with increase in oxygen tension, dilution of nutrient, decrease in number of organisms planted, increase in concentration of certain substances which inhibit growth, and decrease width of bands (NaCl, KCN, CO₂, sulfanilamide), on addition of apparently sub-inhibitory concentrations of methylene blue (3 mg. dye per 1000 cc. — a hydrogen acceptor in specific organismal systems). It decreases for reverse reasons and for certain organisms on addition of 0.5 per cent cysteine hydrochloride or 0.5 per cent sodium lactate. Width of spectrum of certain organisms increases with decrease of nutrient, increase in time of incubation, decrease in organisms planted (with such decrease delimitations and spectrum gradually disappear and one sees discrete individual colonies), or inclusion of bacterial growth products. It decreases for reverse reasons on addition of 0.5 per cent cysteine hydrochloride or 0.5 per cent sodium lactate, on increasing concentration of certain inhibitory substances, on increase of reducing effect below as shown by methylene blue (this in part is dependent on density of band and rapidity of its formation disappearing with increase in discreteness of organismal colonies, decrease in number planted, increase in width of spectrum or growth distribution). Diffusion of growth into medium below is especially marked for certain organisms on inclusion of 1 per cent dextrose or 0.5 per cent monobasic sodium phosphate.

Consistent results are obtainable on planting 0.1 to 0.3 ml. of 24 or 48 hour broth cultures to shakes. Reducing the number of organisms planted reduces delimitation, increases width over which growth occurs and results in discrete and proportionately fewer organisms per unit area. The size of colonies varies, being usually smaller on increase in depth, but sometimes also smaller above. Changes are dependent on the organism: we have been unable to grow certain organisms in bands even on planting large numbers.

On inclusion of methylene blue in the medium, as solidity of the band decreases reduction becomes less marked to absent, and less persistent on standing in the refrigerator when once established. Physiology of growth can be studied by observing reduction areas, concentration of growth in growth bands and oxidation of dye in bands to azures. Concentration of dye frequently occurs just above reduction with a band of white growth below. Several lines of concentrations of varied color may be noted in a spectrum.

While the amount of growth apparently decreases in most instances with decrease in nutrient, time of initiation of growth often is more rapid in lower concentrations (6 hour incubation).

Controls grown in the atmosphere show similar tendencies, but not as well demarcated changes, even after long incubation. Often successive layers of growth appear in time both in controls and under increased oxygen pressure. The superficial layers apparently prepare a lower depth for growth. This does not seem the only cause of bands, since they are observable in certain 6 hour cultures separated by a clear space. The latter suggests oxygen tension variants.

Applications to pathology and bacteriology are: (a) correlation of effects of

The agglutination tests when performed by a competent worker under standardized conditions give prompt and strongly positive results, and are not "temperamental" but clear-cut and of diagnostic value, although negative results do not exclude Weil's disease, and an atypical, delayed and partial serological reaction may or may not be Weil's disease. Unfortunately the sample serum that has been taken from the patient during the last of his illness or after he has recovered from the disease comes to us too late for animal inoculation and cultural tests. Our positive findings, with a few exceptions, are based on agglutination and lysis tests, although we prefer to make the diagnosis on the basis of animal inoculation (with blood and urine) and cultural tests, and confirm it by agglutination tests. The studies are still in progress. We have strains of *Leptospira* with very low virulence for guinea pigs. Some strains fail to produce any demonstrable infections in guinea pigs at all, yet these strains are highly infective and fatal to certain species of American deer mice, *Peromyscus eremicus*, *Peromyscus maniculatus*, and so on.

In reply to Dr. Furth's question, I have had no experience as to X-ray effects on mice (*Mus musculus*) as far as leptospirochetosis is concerned, but I have a definite proof that *Mus musculus* (house mice, "laboratory white mice") and also certain species of field mice and rats, are carriers of *Leptospira icterohaemorrhagiae*.

I should like to state that during 1933 in St. Louis, Missouri, I found *Mus musculus* ("laboratory white mice") naturally infected with *Leptospira icterohaemorrhagiae* and *Spirochaeta morsus muris*. This I found accidentally while attempting to transmit *Trypanosoma duttoni* infection into *Peromyscus* (American deer mice). At that time, to my astonishment, the field mice came down with fatal leptospirochetosis and died within 3 to 5 days. At the height of this infection their blood was swarming with *Leptospira*. This accidental observation gave us a new laboratory animal for experimental leptospirochetosis. I have confirmed this finding on many occasions and have used several hundred field mice during the last 5 years. This fact was mentioned by me in Science, N. S., February, 1935. However, this is the first time that I have officially announced these new laboratory test animals (*Peromyscus eremicus*, *Peromyscus gambeli*, and so on) for studying *Icterohaemorrhagiae* spirochetosis.

BACTERIAL GROWTH "SPECTRUMS." II. THEIR SIGNIFICANCE IN PATHOLOGY AND BACTERIOLOGY. John W. Williams, Cambridge, Mass.

Abstract. Production and application of growth spectrums are discussed elsewhere (*Am. J. M. Technol.*, May, 1938). They are well demonstrated in shake cultures containing a low concentration of nutrient (as 2 to 0.1 gm. nutrient broth Difco per 1000 ml., pH 5.2 to 7.3) when grown for 48 hours at 37°C. under a pressure increased to 60 pounds with 99.5 per cent oxygen. Since increase in concentration to 30 gm. agar per 1000 cc. tends to increase turbidity of medium and decrease growth, 7.5 gm. is preferable. The amino acids (1 per cent) dl-alanine, d-arginine or d-glutamic acid as source of nutrient, produce good growth spectrums in most instances.

The organisms studied were *E. coli communis*, *B. subtilis*, *Staphylococcus aureus*, *Sarcina lutea*, *Serratia marcescens*, *Ps. fluorescens*, *Ps. aeruginosa*, *Acrobacter acrogenes*, *B. megatherium*, *Proteus vulgaris*, *B. niger*, *B. mycoides*, *Staphylococcus albus*, *Proteus X 19*, *B. anthracosis* (non-virulent), and *S. enteritidis*.

stance with increase in its concentration in the various tubes by noting variation in the band, its depth and width at one incubation and one can furnish a pictorial record. I know of no method by which similar results can be obtained as conveniently and with as great satisfaction with the substances and organisms I have studied.

THE EFFECT OF CIRCULATING TOXINS ON THE LUNGS. Douglas H. Sprunt and (by invitation) C. W. Camalier, Jr., Durham, N. C.

Abstract. Fifteen rabbits were injected intravenously with small amounts of staphylococcus toxin. The lungs of these animals showed focal cellular accumulations both in the interstitial tissues and in the perivascular lymphatics. These cells were after 24 hours predominantly mononuclear. The smaller blood vessels also showed some damage.

Eighteen rabbits, which were given larger amounts of toxin and which either died or were killed within 24 hours, showed damage to the arterioles with focal areas of hemorrhage and cellular proliferation. The cells were both polymorphonuclear and mononuclear.

The lungs from the animals of both of these groups were sterile on culture and no bacteria were seen in histological preparations.

In addition to these experiments, several autopsies revealed an abscess as a focus of infection and a non-bacterial interstitial mononuclear pneumonia. In these cases the vascular endothelium showed no appreciable damage.

A few of the experimental animals showed a change in the bronchi and bronchioles which we believe is of importance. This change in many instances is an edema which pushes up the bronchial cells from the basement membrane. In some instances there is a necrosis of these cells and in others a cellular infiltration. These lesions are important as they afford a means of entry for bacteria and the production of a secondary pneumonia.

THE EXPERIMENTAL PREVENTION OF SILICOSIS BY METALLIC ALUMINUM. Dudley A. Irwin, Toronto.

Abstract. It has been found that *in vitro* the presence of small amounts of metallic aluminum renders quartz and silicate dusts practically insoluble. The lungs of rabbits exposed to quartz dust containing 1 per cent metallic aluminum showed only a mild foreign body reaction up to periods of 1 year. Control rabbits exposed to quartz dust alone developed acute silicosis in 6 months.

Discussion

(Dr. Wiley D. Forbus, Durham.) I should like to ask Dr. Irwin if he has any information relative to a change in the ability of the phagocytes to pick up this material when it is in its pure form, and after the aluminum is added.

(Dr. James Ewing, New York City.) Is this method necessary if Kelly's mask is used by the men working in these dusts, or is there any way of combining these agents?

(Dr. Theodore J. Curphey, New York City.) Will exposure to aluminum dust alone stimulate the macrophage reaction of the lung?

(Dr. Irwin.) In answer to the question as to whether the phagocytes of the lung are more active in engulfing the quartz particles when aluminum is present, we do not know of any evidence we can submit to support this view. When

bacteria *in vitro* and *in vivo*; (b) effects of drugs, and so on, on spectrums (as aid in classification of their action: opposite effects of sulfanilamide and sodium lactate on the spectrum may explain why sodium lactate combats the overdosage effects of sulfanilamide in the body, change of oxygen-medium relationship at which the organism grows best may reduce its harm to tissues); (c) study of antagonisms and synergisms; (d) study of substances inhibiting and promoting bacterial growth; (e) determination of suitable synthetic mediums; (f) differentiation of organisms, *i.e.* by spectrums, effects of substances on, ability or inability to grow as bands, and so on; (g) growth of anaerobes after preparation of area below by aerobes; (h) probable oxygen tension variants; (i) study of growth limiting and growth spreading factors *in vitro* with possible correlation *in vivo*; (j) possible identification of hydrogen donators and hydrogen acceptors with respect to specific organismal systems; and (k) study of redox and pH by dyes. While our study of the above applications is limited to a limited number of organisms, conditions and substances in a limited number of concentrations, each has showed a variable degree of promise, depending on the organism.

Discussion

(Dr. M. G. Sevag, Philadelphia.) Those who have had experience in bacterial respiration know the complexities of the bacterial growth systems. I think in such a system one is not able to correlate various factors and their interaction by means of spectrum analysis. Such systems could be more satisfactorily studied if the organisms were isolated free from media, and their activity studied in simpler systems. By taking the results of such studies one can formulate the broad principles for the study of complex systems manifested by the actively growing organisms. For example, during the growth of pneumococcus or streptococcus, the hydrogen peroxide formed inhibits the growth. Catalase, on the other hand, will accelerate growth by removing the inhibiting hydrogen peroxide. One can prevent the accumulation of such inhibiting or growth promoting factors confining studies to the mechanism of metabolic activities. I think it would be more advantageous to deviate from antiquated procedures and study the mechanisms of the simple systems of the physiological activities of organisms. Many isolated facts have been obtained on bacterial enzyme activities; these studies, however, are mainly carried out by biochemists and chemists to elucidate certain theoretical aspects of biochemical reactions. The accumulated results of their studies, unfortunately, so far have not been used sufficiently in the correlation of various factors in explaining many complex immunological and bacterial growth problems. I should like to call to the attention of the workers in this and in related fields this fundamental point of view. I think better results could be obtained by the study of this phase of the problem. By going into the core of the elementary steps, one is able to explain complex systems such as presented by Dr. Williams.

(Dr. Williams.) I believe there is some misunderstanding as to the complexity of this method. While we use a comparatively simple chamber, possibly the even simpler pressure cooker might be adapted to hold 60 pounds the required period. In addition, a tank of commercial oxygen, possibly an incubator, a folding camera, a lens so that the camera can take a close-up and a source for transmitted light are needed. By making 60 to 70 exposures to the film the cost per photograph runs about one-quarter cent. One has a permanent record and can eliminate to a large degree the personal equation. For example, one may trace the course of inhibition of a drug, an antiseptic or some other sub-

greater resistance develop a disease of longer duration, characteristic of the so-called childhood type with caseous pneumonia, massive enlargement and caseation of the tracheobronchial nodes and large nodular lesions of hematogenous origin in different parts of the body. The most resistant family dies on first exposure from a disease of long duration, which is characterized by a localized, isolated ulcerative pulmonary phthisis in which the tracheobronchial nodes and the rest of the body are not grossly affected and which is anatomically indistinguishable from adult pulmonary tuberculosis in man, which has hitherto been considered as resulting solely from a reinfection.

By various procedures the following are among the genetic factors that have thus far tentatively been shown to be related to the resistance of these rabbit families to tuberculosis.

1. The primary sensitivity of the tissues on first contact with heat-killed tubercle bacilli. The greater the primary toxicity of the tubercle bacillus for the skin of an animal, the greater is the susceptibility of that animal to tuberculosis.

2. The acquired sensitivity of infection. All else being equal, a higher allergic sensitivity acquired during infection is associated with a greater resistance to tuberculosis and, conversely, rabbits that develop the least sensitivity to tuberculin are often most susceptible to the disease. The degree of allergy of infection is itself partly determined by the innate sensitizability or responsiveness of the skin to substances in heat-killed tubercle bacilli.

3. The permeability of the skin to particulate matter. The greater the permeability of the skin of a rabbit to india ink the greater is the susceptibility of that rabbit to tuberculosis. None of these factors alone accounts for resistance but their interaction and doubtless the collaboration of many other factors determine resistance.

4. An analogy has been found between the mode of reaction of artificially immunized and naturally highly resistant rabbits. In both instances the reaction to the tubercle bacillus is accelerated and abortive as compared with the reaction on first infection of rabbits of low inherited natural resistance.

Discussion

(Dr. Joseph Kasper, Detroit.) I should like to ask Dr. Lurie if he has made a comparison of the patency of the lymphatics in his various groups of animals. The reason for asking is that on occasion we have noted in the tissues of certain children who showed spreading of their tuberculous lesions an increased patency of the lymphatics in the regional lymph nodes. We have also noted that adult negroes sometimes show the same changes.

(Dr. Louis L. Dienes, Boston.) A few years ago a very interesting study was published by Verschuer and Diehl in Germany concerning the influence of inherited constitution on the development of human tuberculosis. By comparing the incidence of tuberculosis in identical and non-identical twins they observed that in the case of identical twins both usually develop tuberculosis, while in the case of non-identical twins it is relatively rare that both are tuberculous. Similar studies might be of great importance in making possible the application of results obtained by animal experimentation to the problems of human tuberculosis.

I should very much like to know Dr. Lurie's impression of the observations of Verschuer and Diehl.

aluminum is present the phagocytes stain so well we think it is evident that whatever the toxic material produced by the quartz may be, it is not present in sufficient concentration to cause the death of these cells.

In answer to Dr. Ewing's question, we hope that a dust mask may be designed which will remove all the dust from the inspired air, but so far we have not been able to find such a mask applicable to the thousands of underground workers in gold mines. We are not suggesting that the use of metallic aluminum will do away with masks or the best ventilation possible, but we do think it would be advantageous to add aluminum to the dust to inactivate any quartz that might get into the lungs in spite of dust control.

The lungs of animals exposed to aluminum dust for periods of a year contain a fair amount of the dust. The aluminum dust is engulfed by phagocytes in the alveolar spaces of the lungs and what seems odd to me is that very little aluminum is found in the peribronchial and mediastinal lymphatics. Two months after discontinuing the aluminum dust much of it has disappeared from the lung.

THE TUBERCULIN REACTION AND ITS SIGNIFICANCE IN LEPROSY. Earl B. McKinley, Washington, D. C.

Abstract. It has long been known that a high percentage of cases of leprosy give positive tuberculin reactions. Recent controlled studies have been made in the Philippines with antigens from human, bovine and avian strains of *M. tuberculosis*. Groups studied included (1) the children of lepers; (2) controls — children having had no contact with lepers; (3) advanced cases of leprosy; (4) early cases of leprosy; (5) preparole cases of leprosy following treatment; (6) families of lepers; and (7) professional contacts — physicians, nurses and so on. The problem of tuberculosis in leprosy is discussed and the significance of the tuberculin test in this disease is critically analyzed.

Discussion

(Dr. Esmond R. Long, Philadelphia.) Was the character of the skin reaction as given by the proteins from so-called leprosy bacilli comparable to that given by the protein from the tubercle bacillus?

(Dr. McKinley.) Yes, and I might add that at least 90 per cent of the reactions are what we would call ++, the other 10 per cent being spread largely in the +++ group, with very few in the ++++ group.

THE RÔLE AND NATURE OF INHERITED NATURAL RESISTANCE TO TUBERCULOSIS. Max B. Lurie, Philadelphia, Pa.

Abstract. By brother and sister mating of rabbit groups, families have been developed that exhibit varying inherited specific resistance to tuberculosis. This has been determined by natural respiratory exposure to artificially infected rabbits and by parenteral administration of standard quantities of tubercle bacilli. Irrespective of the mode of infection it was found that by excluding all known environmental factors the genetic constitution of the rabbit by itself may determine the type of disease developed on first natural contact with the tubercle bacillus. Those hereditarily most susceptible develop a disease of short duration, characteristic of infantile tuberculosis with miliary spread from a primary pulmonary-glandular complex. The animals of somewhat

dietary conditions. We keep them on the same basic diet that Goldberger used to produce pellagra, and after observing them for a while we introduce one substance after another and test the therapeutic effect. From my analysis of the diet I think it is probably deficient in riboflavin, but we have no simple way of measuring it. Could we use a slit lamp to test for this deficiency?

(Dr. D. Murray Angevine, New York City.) I should like to ask if there were any changes noted in other structures of the eye.

(Dr. Wolbach.) In answer to Dr. Smith's question, these vessels may be readily seen by the slit lamp. We used that method in the hope that we might pick up some antecedent change in the corneal epithelium. It is an entirely practical method to apply to patients with riboflavin deficiency.

In answer to Dr. Angevine, if there were other lesions in the eye, nothing of importance was found; in all deficiencies, as is common with any starvation, there is atrophy of the glands associated with the eye, the harderian gland and the lacrymal gland, but in the remaining tissues we found nothing. However, there may be something there.

(Dr. Smith.) May I also ask if the reaction is reversible and disappears with riboflavin treatment?

(Dr. Wolbach.) I should have said more about that. Repair is extremely rapid. It is so rapid that a very striking change takes place in the cornea. Repair is so rapid as to indicate that perhaps water has something to do with it. There is a marked water retention during the repair stage.

THE CYTOLOGY OF THE HUMAN PARATHYROID GLANDS. Edgar H. Norris, Minneapolis, Minn.

Abstract. The observations made in this investigation are based on the histological study of 1500 parathyroid glands—406 glands from the fetal period and 1094 normal or pathological glands from postnatal life.

In the literature the terminology as applied to parathyroid cytology is regrettably confused and comprehension of an author's intended meaning is now little more than speculation. Although we should have little patience with most efforts to engender new names, the advantages of the adoption of a uniform terminology to be applied to the cytological elements of these glandules are self-evident. Part of the present confusion has come about because the terms in use have carried only morphological significance. Another part of our difficulty has arisen from the fact that some investigators have not recognized the same number of distinct types. This then is the problem, and inasmuch as the suggestions about to be made appear both to clarify and to simplify, an arrangement is presented that may be of practical use. The value of our scheme comes from the fact that, in its development, morphological, genetic and functional features have been accorded equal importance. Established terms have been preserved where they are morphologically significant and all of the terms have been rigidly defined. Five discrete cytological types are recognized and in addition there appear to be 5 transitional forms that merit special denomination. The terms recommended are primordial cell, dark cell, large primordial cell, small vesicular cell, large vesicular cell, clear cell, and oxyphil cell, of which latter small or large forms of both the pale and the dark types are noted.

The term primordial cell is recommended for several reasons. In the first place the primordium of the parathyroid in the embryo is made up entirely by

(Dr. Lurie.) As to the patency of the lymphatics, I cannot answer that question as we have not done any direct investigation on this as yet. I do not know whether that would be a significant factor. It may be. The point is this. When you inoculate these animals that are so highly resistant to tuberculosis they die from a disease of long duration, but the lymph nodes draining the site of inoculation become caseous. However, if they acquire the disease by exposure they develop a localized ulcerative phthisis in which the draining tracheobronchial lymph nodes are not affected, a disease anatomically indistinguishable from adult pulmonary tuberculosis in man.

As to Dr. Dienes' question about the work of Diehl and Verschuer, I have read that very carefully and I think on the whole their conclusions seem to be sound. However, there is one point that cannot be overlooked — no such studies can ever definitely answer these questions in human beings because of environmental factors which cannot be wholly excluded. The work has been criticized by B. Lange. He emphasizes that the very identity of the twins makes them live under similar conditions to a greater extent than non-identical twins, and it is conceivable that identical twins have the same psychology, and therefore they would react similarly to the same conditions. In fact, an actual analysis of the work shows that the environmental conditions in these identical twins were more often the same than the environmental conditions of non-identical twins, so that while I believe in and approve of the conclusions, I do not think the question can be settled in any other way than by animal experimentation where environmental factors can be completely excluded.

VASCULARIZATION OF THE CORNEA OF THE RAT IN RIBOFLAVIN DEFICIENCY.

Otto A. Bessey (by invitation) and S. B. Wolbach, Boston, Mass.

Abstract. In rats maintained on a diet deficient only in riboflavin, vascularization of the cornea is a constant phenomenon. The initiation, progress and repair of this process have been followed by histological sections, injected and cleared specimens, and by slit lamp observations.

We have been unable to find any antecedent process in the cornea and therefore believe that the vascularization is a response to a nutritional defect, presumably of the tunica propria.

Following vascularization, opacity of the cornea appears and histologically it is accompanied by infiltration of leukocytes and subsequently striking changes in the collagen. Lesions of the corneal epithelium appear to be secondary to the degenerative changes in the tunica propria. Ulceration is a late consequence.

Repair following administration of riboflavin (synthetic) is rapid. Except in late lesions, the opacity of the cornea clears in from 12 to 48 hours. The order of sequence in repair is apparent restoration of the collagenous substance of the tunica propria, disappearance of leukocytes, repair of corneal epithelium and disappearance of blood vessels. The whole process is complete in about 10 to 14 days.

Primary vascularization of the cornea is apparently specific for riboflavin deficiency. Its initiation and repair have been advantageously used for testing the biological activity of a number of compounds structurally related to riboflavin.

Discussion

(Dr. David T. Smith, Durham.) At the Duke Hospital in Durham, N. C., we have a number of patients with pellagra in the hospital under controlled

the question of whether these cells are derived from the same or different precursor cells. However, after they have developed from whatever source, the lack of effect of estrone on thyroidectomy cells when injected in a dosage adequate to suppress castration cells, but not in such large dosage as to degranulate all cells, is a further argument that these cells are functionally different cells and may represent cells that are producing different hormones.

A HISTOLOGICAL STUDY OF THE EFFECT OF THE SEX HORMONES ON THE HUMAN PROSTATE. Robert A. Moore and (by invitation) Allister McLellan, New York City.

Abstract. During the past year several clinics have undertaken to treat benign hypertrophy of the prostate by injections of the male or female sex hormones. Aside from the disputed clinical efficacy of these forms of therapy, which will not be discussed in this report, failure in some cases has given the pathologist an opportunity to study the histological appearance of the prostates of patients given large amounts of the sex hormones.

The present study is concerned with 10 unselected consecutive cases of benign hypertrophy of the prostate; 5 individuals were given from 400 to 1075 mg. of testosterone propionate for from 16 to 95 days before a prostatectomy, and 5 were given from 15,000 to 120,000 international units of estradiol benzoate for from 12 to 32 days before a prostatectomy.

In those patients given testosterone propionate there is no significant change in the structure of the presenile prostate, and it is concluded that the presenile and senile involution is not the result of a simple decrease in the secretion of androgen by the testis. There is also no deviation from the usual appearance of the tissues which are a part of benign hypertrophy.

In those patients given estradiol benzoate there are conspicuous changes in the urethra and prostatic ducts. There is hyperplasia of the epithelium and metaplasia to a squamous type. There is advanced migration of leukocytes through the epithelium. About the ducts there is abundant lymphoid tissue. Aside from rare foci of squamous metaplasia there are no changes in the appearance of the epithelium or stroma of the nodules seen in benign hypertrophy.

Conclusions concerning the significance of these findings would be premature, but as a basis for further work it is important to note that the changes seen after injection of estrogen are an exaggeration of some of the characteristic features of benign hypertrophy.

THE INDUCTION OF CARDITIS BY THE COMBINED EFFECTS OF HYPERTHYROIDISM AND INFECTION. Mark P. Schultz (by invitation), Washington, D. C.

Abstract. Observers do not agree upon the occurrence of extensive morphological cardiac damage in patients with exophthalmic goiter or in animals given toxic doses of thyroid hormone. Study of published protocols and case records suggests that such lesions may occur when hyperthyroidism is complicated by infection. Thirty-two rabbits and 13 guinea pigs were given moderate doses of thyroxin or desiccated thyroid while subject to chronic, focal hemolytic streptococcus infection. Most of them developed extensive non-purulent carditis similar to that occasionally described in exophthalmic goiter and in animals treated with thyroid hormone. Such results were obtained in those infected after hyperthyroidism had been induced; no cardiac lesions developed when thyroid hormone was given after infection had become well established. This

cells of this type, and in the second place it is from cells of this type that all of the other parenchymal cytological elements are derived. On the other hand, this term is recommended to take the place of the names chief or principal cell, which are often misleading, because in many glands this type does not predominate.

Cytological pictures made up of large primordial cells or of cells with increasing degrees of vesiculation are believed to represent increased functional activity. As yet there is no knowledge regarding the functional significance of the oxyphil cells.

Discussion

(Dr. W. G. MacCallum, Baltimore.) I do not think I have anything to add, except that the clear cells are the ones we find in the early stages of development, and that they predominate, or are the only ones found, so far as I can say, in an infant's parathyroid. As to the exact relation of these to the so-called primordial cells, I have always had the idea that these primordial cells were developed from the clear cells. I have no real data, and I think that the eosinophil or oxyphil cells are variants from that point. I think it would be extremely interesting to find some more specific stains for these cells, just as we have the same difficulty in the adrenal cortex in finding specific stains for cells. It would be important in determining the functional character of such cells.

(Dr. Benjamin Castleman, Boston.) I should like to ask what led Dr. Norris to think the dark cells are inactive. It seems to me we have seen active tumors of this gland that have had what he calls a dark cell.

(Dr. Norris.) I am afraid I cannot answer these gentlemen very definitely. Dr. MacCallum's idea is perfectly correct. In my experience the parathyroids in childhood are definitely constituted by what we call vesicular cells of the small type, and it is our feeling that probably the parathyroid may be considered to be functionally very active during that period.

One reason for the use of the term "primordial" is on the basis you implied; not in childhood but in the embryo we find this type of cell so characteristically making up the parathyroid.

In answer to Dr. Castleman our evidence is only that of rationalization. We find this dark type of cell only infrequently, and it appears to be at the other end of the morphological line from the cells we have considered as evidence of hyperactivity.

FURTHER EVIDENCE THAT "CASTRATION CELLS" AND "THYROIDECTOMY CELLS" ARE DIFFERENT TYPES OF PITUITARY CELLS. Isolde T. Zweckwer, Philadelphia, Pa.

Abstract. The author has previously described histological differences between the cells in the pituitary that develop after thyroidectomy and the cells that develop after castration in rats 11 weeks or more after operation. A number of investigators regard the cells as identical.

The experiments here reported concern the differences in histological appearance of these cells at 5 weeks after operation. At this earlier period the differences are more apparent than later. Furthermore, the testes do not atrophy, but may hypertrophy, so that thyroidectomy cells cannot be due to lack of hormones from the gonads. The differences in response of thyroidectomy cells and castration cells to the administration of estrone and of thyroid extract prove that functionally these cells are totally different. It seems impossible to solve

roidism suffers damage incident to infection which would otherwise not cause such an effect. The use of exercise in conjunction with hyperthyroidism also is no doubt a factor. We thought this might have been ruled out in part by the failure to observe a potentiating effect when treatment with adrenalin was combined with infection.

PATHOLOGICAL EFFECTS OF ELIXIR OF SULFANILAMIDE POISONING. Paul R. Cannon and (by invitation) Eugene M. K. Geiling, Chicago, Ill.

Abstract. Autopsy material was examined from 14 fatal cases of poisoning by elixir of sulfanilamide (Massengill). The patients varied from 11 months to 70 years of age, and had taken from 1.5 to 6 ounces of the elixir. All developed symptoms of abdominal distress, nausea, backache, suppression of urine and uremia. Complete autopsies were not performed in most instances, but the principal changes were in the kidneys and livers. The material was sent to us by Federal inspectors and pathologists.

The kidneys and livers in patients dying from the poisonous action of the elixir of sulfanilamide were edematous. The kidneys were usually pale and frequently had diffuse areas of symmetrical cortical necrosis with a zone of hemorrhage at the corticomedullary border. The liver was usually mottled and the centers of the lobules were lighter than the peripheries. The kidneys microscopically were most affected in the convoluted tubules where there was a profound vacuolization or hydropic degeneration with obliteration of the lumens. The cytoplasm of each cell was ballooned out and the nucleus was shrunken and pyknotic. Fatty changes were minimal and actual coagulation necrosis was inconspicuous, except at the margins of the affected areas and in the zones of cortical infarction. Here there was complete lysis of the tissue in places and recent diffuse hemorrhage into the interstitial tissue. The blood vessels were filled with hyaline thrombi, and at times the arterioles were severely affected by hyaline necrosis of the wall, which at times extended into the afferent arterioles of glomeruli. Hyaline casts were numerous in the collecting tubules but the epithelium here was but slightly changed, although showing moderate fatty degeneration. Oxalate crystals were not found in any of the areas of hydropic degeneration or elsewhere. The changes in the liver were mainly a hydropic degeneration around the central veins where the hepatic cells were swollen and vacuolated, with shrunken nuclei. The vacuoles did not contain fat. The hepatic cells at the margins of the areas of central hydropic degeneration were essentially normal and contained no increase in number of mitotic figures or double nuclei.

The changes elsewhere were essentially those that might be due to uremia and acidosis. There was frequently an accumulation of clear fluid in the peritoneal, pleural and pericardial cavities. Pulmonary edema was usually present at the time of death, and bronchopneumonia also, in some cases.

We observed practically identical changes in dogs, rats and rabbits fed with comparable doses of elixir of sulfanilamide or pure diethylene glycol, given by mouth in divided doses. The cortical infarcts of the kidneys did not occur in the experimental animals, but otherwise the pathological changes were those of hydropic degeneration of the convoluted tubules and central hydropic degeneration of the liver. Sulfanilamide alone, in large doses, caused no such effects; it only caused a mild degree of fatty degeneration of the tubular epithelium at the corticomedullary border.

suggests that for the production of such lesions it is necessary that the host experience an alteration in immunological reactivity during the course of hyperthyroidism. Experiments are described which indicate that immunological responses are accelerated in hyperthyroidism. The hearts of animals receiving thyroxin or dried thyroid only in the doses used, or subject to infection only, did not present such lesions. Likewise, dinitrophenol substituted for thyroid hormone was ineffective.

Discussion

(Dr. Joseph D. Aronson, Philadelphia.) This paper interests me very much, for a number of years ago I had occasion to test cretins with the tuberculin test and not a single one of these reacted to tuberculin. I also attempted to do some skin tuberculin tests on monkeys, which are well known not to react to injections of tuberculin, and in discussing the matter I found the thyroid is rather poorly developed in monkeys. I therefore gave them large injections of thyroxin, but despite that the tuberculin reaction in these monkeys was negative.

(Dr. Howard T. Karsner, Cleveland.) The experiments of Dr. Schultz have been of the utmost interest. I think it would add further to our information if we knew what might happen if something other than, but perhaps similar to, thyroxin were also injected into another series of animals.

The recent work on ascorbic acid by Ecker and others in relation to immune phenomena and complement raises the question as to whether or not the ascorbic acid content of the blood was determined.

(Dr. S. B. Wolbach, Boston.) I wonder if Dr. Schultz recalls Dr. Goodpasture's paper on the same subject. I recall, about 20 years ago in Boston, stimulated by the findings in a patient who died of a thyroid storm following operation, Dr. Goodpasture did some experiments on rabbits and he got acute myocardial lesions following exercise by putting the animals in a treadmill, and also obtained similar lesions by injecting killed bacteria.

(Dr. Schultz.) With respect to the lack of tuberculin hypersensitivity in cretinism, or the possibility of affecting it in monkeys by treatment with thyroxin, it may be pertinent to mention some of our experiments with reference to the development of bacterial hypersensitivity. When rabbits are given minute doses of indifferent streptococci intracutaneously, which under ordinary circumstances cause the development of intense bacterial hypersensitivity, it was found that those treated with thyroxin did not develop large cutaneous lesions; the lesions were, indeed, quite small, as were those described in thyroxin-treated animals injected with horse serum intracutaneously.

It is of great interest to attempt to analyze these phenomena from the standpoint of metabolic factors since the replacement of thyroxin by dinitrophenol in infected rabbits does not result in the induction of cardiac lesions, although the metabolic rate is probably elevated to an equivalent degree.

Dr. Karsner's observation concerning ascorbic acid is most pertinent in view of the well known antagonism between ascorbic acid treatment and treatment with thyroxin. We had this in mind when these experiments were performed and took care to see that each animal received at least 40 gm. of cabbage daily. Since 5 gm. of cabbage daily is considered sufficient to prevent the development of scurvy in such animals we felt that protection against this deficiency disease had been accomplished.

With respect to Dr. Goodpasture's experiments some years ago, a notably increased susceptibility to liver damage from chloroform anesthesia was observed. Most recently, Habán has demonstrated that the liver in hyperthy-

Dr. Schmeisser has described, either in human material or in animals, but we have in the kidneys.

Inasmuch as our material came from incomplete autopsies, we have seen sections of suprarenals in but 1 case and here no pathological changes were apparent. In some of the animals given diethylene glycol we have seen considerable vacuolization of the cells of the cortex, but in only an occasional animal.

UTERINE ADENOMAS IN THE RABBIT. Harry S. N. Greene and (by invitation) John A. Saxton, Jr., Princeton, N. J.

Abstract. Ninety-five cases of an adenomatous tumor of the endometrium have been found in a colony of approximately 500 female rabbits during the past 4 years. The presence of tumor was noted during life in the majority of instances and the clinical course led to death or disposal. Pathological studies were made at various stages of growth, and both homeotransplantation and heterotransplantation of the tumor were successfully carried out.

The clinical histories of tumor-bearing animals are similar in all cases. A long period of reproductive disturbance precedes discovery of the tumor and its subsequent course is one of slow progressive growth which has terminated in death with metastases in all animals held under observation for more than 1 year.

The tumor shows an atypical alveolar structure and its characteristics closely resemble those of an adenocarcinoma of the uterine fundus in women. Pathological changes similar to those found in mice after treatment with estrogenic substances occur in the adrenal, thyroid, pituitary and mammary glands of affected animals.

Intraocular transplantation has been successful, and at the present time the growth has been carried through 8 generations by serial transfer. Intratesticular transplantation resulted in takes after the 5th eye transfer and growth has been obtained in the 5th serial passage, using this route of inoculation. Heterotransplantation has also been successful and progressive growth of the transplant has been obtained in the eye of the guinea pig, the rat and the goat. Serial transfer has been attempted in the case of the guinea pig, and at the present time growth has been obtained in the 2nd serial generation.

Discussion

(Dr. Max Lurie, Philadelphia.) Do these adenomas occur in certain breeds only, or do they occur generally in many breeds?

(Dr. Greene.) The incidence of the tumors is highest in the same breeds and lines of animals in which the incidence of toxemia of pregnancy is greatest. They occur in all of the common breeds with the exception of the Belgians, Chinchillas and French Silvers. Many of the tumor-bearing animals are closely related and instances of the tumor in 3 generations of a family are of common occurrence.

CLINICAL AND GENETIC OBSERVATIONS ON SPONTANEOUS MAMMARY CARCINOMA OF THE RABBIT. Louise Pearce and Harry S. N. Greene, Princeton, N. J.

Abstract. In a group of interrelated families of Belgian rabbits which comprises one of the pure breeds in a large rabbit breeding colony, the incidence of breast abnormalities, including tumor formation, and of malignant tumors in particular, has been high. Spontaneous mammary gland tumors of the rabbit are gen-

We conclude that the injurious effect of the diethylene glycol in the elixir of sulfanilamide was due to its action upon the epithelium of the convoluted tubules with a resulting swelling and mechanical occlusion of the lumens. As a result, anuria, acidosis and uremia followed. The circulatory changes are probably secondary to the irritation and anoxia produced by the poison and the hydropic degeneration.

Discussion

(Dr. E. T. Bell, Minneapolis.) I should like to call attention to the fact that these are examples of tubular disease, which is very rare in clinical experience, aside from the kidney in sublimate poisoning. The symptoms that develop from tubular disease are anuria and oliguria. There is a very marked distinction here between this disease and lipoid nephrosis, which is often called tubular disease and is characterized by the prominence of edema. Lipoid nephrosis is a glomerular disease, due to loss of protein in the urine, and is by no means a tubular disease.

(Dr. Harry C. Schmeisser, Memphis.) I had the opportunity of studying the pathological changes in 2 cases of elixir of sulfanilamide poisoning which came to autopsy. The findings in both cases were similar and resembled those presented by Dr. Cannon very closely, except possibly that some of the changes were more advanced, leading to necrosis. The livers were enlarged, weighing 2600 and 2130 gm., suggesting a diffuse degeneration. The kidneys were enlarged, weighing each 300 gm. in the one case and 230 and 220 gm. in the other. Numerous petechial hemorrhages were found on the surface with several extensive areas of hemorrhagic necrosis of the cortex. These measured from 1 to 5 cm. in diameter. The medulla did not present any gross evidences of involvement. Numerous petechiae were seen in the renal pelvis. Microscopically the livers showed extensive fat metamorphosis and necrosis of the hepatic cells around the central vein of the anatomical lobule with some extravasation of blood. The kidneys showed marked necrosis of the glomerular tufts and convoluted tubules with extensive hemorrhagic extravasations, hydropic degeneration of the tubular epithelium, and early infiltration of the necrotic tissue by polymorphonuclear leukocytes. The elixir taken by one of the patients was proved to be toxic by the fact that intraperitoneal injections of 5 cc. into rats weighing 200 gm. caused death within 6 hours.

(Dr. Chester, New York City.) I wonder if Dr. Cannon had the opportunity of studying the adrenals from any of these patients. In some of the rats and rabbits we were studying we found the same sort of hydropic degeneration in the cortex of adrenal cells in occasional instances, and I wondered if the same thing had been observed in any of the human material.

Perhaps one other point that might be mentioned in justice to the legitimate usage of the drug is that it apparently takes a reasonably large dosage of the drug to produce these toxic effects. Rats can be kept on 0.5 to 1 per cent in the drinking water for as long as 6 months without any apparent effect, and rabbits can also be on 0.5 to 2 per cent in the drinking water without anatomical changes. The rats took as much as 0.3 gm. of pure diethylene glycol and were apparently unaffected.

(Dr. Cannon.) The gross pathological effects as described by Dr. Schmeisser are similar to those described by Barber in dioxane poisoning (*Guy's Hosp. Rep.*, 1934). He described 5 fatal cases of dioxane poisoning from the inhalation of fumes in a factory. We have not seen such necrotizing effects in the liver as

tumors that appear to be malignant. Another F_1 female has developed recurrent cystic changes of the breasts that have been associated with later tumor formation. At present, however, we prefer not to interpret these findings on the basis of the operation of a dominant factor for two reasons. First, it is possible that future observation will show that tumors of the breast or tumors in other locations occur in the lines represented by the male parents. And second, tumors have not developed in several other F_1 females under observation for more than 3 years. It should be mentioned in this connection that observations on the F_2 progeny are now in progress.

The development of these several interrelated families of rabbits constitutes in reality the development of so-called tumor-bearing lines. The material thus made available provides for the first time an opportunity for investigating the etiology of spontaneous malignant breast tumors in this animal species.

Discussion

(Dr. H. Gideon Wells, Chicago.) I want to congratulate Dr. Pearce and her associates. Just as with the original work of Yamigawa, the importance of this consists in getting the disease in an animal which so seldom has it, ruling out a lot of complicating factors from spontaneous tumors, so I think this is going to give us a lot of valuable information which has been difficult to get in an animal as susceptible as the mouse.

(Dr. Harry S. N. Greene, Princeton.) I have examined sections from animals in various stages of tumor development and there are two points relative to their pathogenesis that I would like to amplify. All of the tumors arise in association with cystic breast changes which are microscopically identical with those observed in cystic mastitis in women. The cystic changes occur and regress, but eventually a nodule persists. Microscopically, the nodule shows tissue changes comparable with those observed in fibroadenoma of the human breast. After a variable time epithelial cells invade the stroma of the nodule. Then one of two things may happen. If the animal continues in good physical condition for a sufficient length of time, invasion and metastasis occur. If, on the other hand, the anaplastic change occurs in an old animal, or in one in a poor physical state which persists, invasion and metastasis do not occur despite the local malignant changes. In some instances atypical proliferation of acinar epithelium occurs in a wide area and fibroadenomatous nodules do not appear, but usually the sequence of events proceeds in the manner described.

The second point I should like to emphasize is the similarity of the endocrine changes in animals with breast and with uterine tumors. The adrenals, thyroid and hypophysis are similarly affected in both groups, but the changes in the hypophysis are most marked in animals with breast tumors. This gland weighs between 50 and 70 mg. in normal animals of the Belgian breed, but in tumor-bearing members the weight is increased to from 200 to 300 mg. Microscopically there is a degranulation of all chromophilic elements with a marked increase in the number of chromophobes. Bioassay shows a complete absence of any normal hypophysial hormones. It seems probable, therefore, that both the uterine and the breast tumors may arise on the same endocrinological basis, and that the location of the tumor in the breast in the Belgian breed and in the uterus in other breeds may be the expression of an accessory hereditary location factor.

(Dr. Peyton Rous, New York City.) Have any injections of estrin been made in an attempt to precipitate the condition?

(Dr. Pearce.) Such experiments are in progress.

erally regarded as being extremely rare, and with this notable exception our own experience is in accord with that of others. An analysis of the female population of the colony covering an 8 year period shows that in 115 females representing 16 pure breeds other than the Belgian, and under observation 3 years or longer, there have been 3 cases of malignant breast tumors, an incidence of 2.4 per cent. In a second group of 124 similar pure bred females followed 2 to 3 years, no breast tumors developed. Finally, in 2 groups of mixed hybrids in which the Belgian breed was not represented, comprising 154 females followed for 3 years or longer and 220 observed for 2 to 3 years respectively, no breast tumors developed.

In contrast with these results are the findings in the group of pure bred Belgians. In 14 females under observation for 3 years or longer, there were 6 cases of breast tumors diagnosed microscopically as adenocarcinoma, an incidence of 42.9 per cent, and a 7th case occurred among 25 females under observation for 2 to 3 years. It is noteworthy that there were 2 instances of tumor development in a mother and her daughter. In 2 groups of miscellaneous hybrids, in which the Belgian breed was variously represented, there were 26 females observed for 3 years or over, and 55 for 2 to 3 years respectively. In the former group there have been 4 animals, 3 of which are living, in which breast changes very suggestive of carcinoma have developed; in the latter group there was 1 case of breast tumor which microscopically was diagnosed as adenocarcinoma. This animal represented a backcross generation to her male parent.

The development of the tumors was associated with a cystic mastitis which occurred during the 2nd year of life and while the animals were in active breeding service. The course of the cystic changes was variable and in some cases they were recurrent. Eventually they became persistent and were associated with more or less induration and accumulation of secretion throughout the mammary ducts. Small shotty nodules could be palpated in the breast tissue and their continued growth formed the basis for the tumor. Microscopic examination of tissue obtained at successive biopsies showed typical progressive changes from adenofibroma or fibroadenoma to adenocarcinoma.

The duration of life of 4 uninterrupted cases was 3 years 2 months to 4 years 6 months, and at autopsy metastatic growths in various sites were found in each case. Two animals were killed while the tumor was comparatively small and no metastatic growths were found. Microscopically, these tumors presented the same picture as those of the 4 metastasizing tumors. The 7th animal is still living, and again the diagnosis has been made by microscopic examination of biopsy tissue. Transplantation of 1 tumor has been accomplished for 1 generation by the intraocular route. Intracerebral transplantation of another tumor was successful for 1 generation, the growth taking place in the lateral ventricle, but subsequent transplantations were unsuccessful.

The Belgian breed of rabbits generally presents many difficulties with respect to the raising of vigorous individuals. The stock from which our animals are the progeny was derived from 5 sources. In the attempt to obtain robust and long-lived animals, these lines were variously crossed with the production of a number of closely interrelated families. Tumors have developed most frequently in certain of these families. Little can be said, however, about the complex genetics of the affected individuals except that each of them is the descendant of the same male ancestor.

Two females still living and representing an F_1 generation, that is, the progeny of a tumor-bearing female and a male of an unrelated breed, have developed

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and upon mice when the dye stuff is introduced subcutaneously at regular intervals. There is no important response in mice at the site of injection. In both mice and rats one can follow the effect of the chemical on the liver. There is marked damage to the liver cells, most pronounced at the periphery of the lobules. A certain number of the liver cells are killed and this, in my opinion, is the cause of the proliferation that is present, both in liver cells and in bile duct epithelium. The bile ducts proliferate in order to maintain continuity with the liver cell columns. There may be enough connective tissue response to produce a considerable degree of fibrosis of the liver. Throughout the 12 to 14 months necessary to produce tumors one can see, constantly, evidences of damage and repair. I have every reason to believe that liver cells, severely damaged, do recover. In the course of time there appear in these livers islands of liver columns quite like those we see in human cirrhotic livers. These islands represent cells that are now able to survive in the presence of the chemical. I believe they have acquired a certain amount of resistance. In the subsequent continuation of the experiment the liver cells in these islands continue to show the effects of the chemical, but much less markedly than normal liver cells, and the rate of repair is able to preserve the tissue as a whole. From these islands of regeneration the tumors take origin.

In another type of experiment — the introduction of dibenzanthracene beneath the skin in pellet form — I find equally definite evidence of destructive effect of the agent, but eventually the reparative response gains impetus and a period ensues when the cells are no longer destroyed by the agent. Here again I see the acquisition of local immunity as a stage in the transformation of the cells.

In skin paint experiments, using benzpyrene and methyl cholanthrene, a less easily followed sequence of events indicating acquisition of considerable degrees of tolerance or immunity to the agents used can be demonstrated.

I have no hesitation in saying that the agents I have used have all been destructive in the first effect and since the idea came to me of immunity as one of the stages in carcinogenesis I have been studying the tissues more carefully in order to get an idea of the type of cellular damage produced by the chemicals. The effects of all these agents on the cells reminds me much more of the effects of bacterial toxins. I am now very certain that cells may be severely damaged and yet survive and that we must take into account repair of the cell structure itself as an important feature, and this is to be distinguished by the repair that follows the death of cells that require mitotic division.

It is through this type of study that I get courage to throw the gauntlet to Dr. Rous in regard to his thesis that the unknown agent of tar carcinoma is a virus and that all that tar does is to open the door for the entrance of viruses. I prefer to take the viewpoint that the Shope virus, for example, is merely another carcinogenic agent, and that the transformation of normal tissue to tumor tissue is brought about by the type of activity within the cell which the agent produces providing the responses are maintained over a sufficiently long period of time. A tumor produced by a so-called carcinogenic chemical continues to grow and it has been shown that it eventually shows no trace whatsoever of the agent employed. I am inclined to believe that the carcinomas induced through the action of the Shope virus become quite independent of the presence of the virus. In other words, the virus is needed for a period of time to bring about the irreversible changes, whatever they are, that accompany the genesis of the tumor.

This afternoon I reported on the effect of riboflavin deficiency on the cornea of the rat. I said nothing about the effect of riboflavin deficiency on the skin of

A COMPARISON OF THE VIRUS-INDUCED RABBIT TUMORS WITH THE TUMORS OF UNKNOWN CAUSE ELICITED BY TARRING. Peyton Rous and (by invitation) John G. Kidd, New York City.

Abstract. A comparison has been made of the likenesses and differences exhibited by the papillomas resulting from inoculation of the Shope virus into domestic rabbits and the benign epidermal tumors of unknown cause which follow upon tarring. The virus growths are of a single, sharply defined sort, whereas those evoked by the tarring, though deriving from the same basal region of the epidermis, are of two kinds — the relatively rare “frilled horns” and common papillomas. Many of the latter when stimulated by tar take on the appearance of carcinomas without really being such. They are the well-known “carcinoids” which later, when tarring is discontinued, either revert to the papilloma form, become mere keratinized cysts, or vanish. So distinctive are the characteristics of the frilled horns and tar papillomas as to suggest that they have specific differing causes.

No serological relationship between the virus and the unknown cause for the tar papillomas has been demonstrable, yet histologically the latter are strikingly like the growths caused by the virus when it reaches the epidermis by way of the blood stream. Tarring will enable it to do this, and furthermore to produce growths when otherwise this would not happen. Both the tar papillomas and the virus papillomas in tarred skin arise from the deeper side of the hair follicles; they evolve similarly, grow by intrinsic cell proliferation, and assume the same forms when proliferating, stationary or retrogressing. But the unknown cause of the tar papillomas does not act unless the cells have been changed in some special way by the “carcinogenic” tar, and it exerts only a mild and conditional compulsion. The virus, on the other hand, can act upon cells damaged in many ways; it urges them to vigorous proliferation, and the resulting tumors will progress without the aid of tarring, though markedly stimulated thereby. The tar papillomas do not ordinarily survive transplantation within their host, whereas the virus growths usually flourish. The formative influence of the unknown cause for the tar papillomas is not great, these frequently becoming carcinoids temporarily; whereas that of the virus is so pronounced that the carcinoid form is assumed only under exceptional circumstances. Carcinomas sometimes develop secondarily from tar papillomas after many months, while they arise often and early from virus papillomas, but by morphological changes that are similar. All in all, the comparison has shown that the unknown cause of the tar papillomas makes the epidermal cells do essentially what the virus makes them do, the observed differences being quantitative in nature save as concerns minor details.

Discussion

(Dr. S. B. Wolbach, Boston.) I present my ideas with a great deal of caution because I view the problems of carcinogenesis from a very different angle, which has come from the experiences of long pathological practice and some work I have done on following the responses to carcinogenic chemicals. I was rash enough to talk about this a few years ago. Since then I have accumulated a large amount of additional material but have not yet committed myself to publication.

One of the compounds I have worked with is the dye stuff, 2-amino-5-azotoluene, first shown by Yoshida to produce liver cell carcinomas after prolonged feeding to rats. I have studied the effect on rats through feeding experiments

Zoological Garden. Thus far, lesions have been limited to the pancreases of snakes (*Serpentes*). Of 72 specimens from both old and new world species, 15 have exhibited histological changes that suggest carcinoma. Focal lesions, possibly early stages in the process, were found in 9 others. As a rule, both types of abnormalities can be detected only by microscopic study. Neither metastasis nor extension to other organs has occurred.

The exact nature of these lesions cannot yet be determined, but it is possible to rule out postmortem artefacts and response to acute inflammation.

Discussion

(Dr. H. Gideon Wells, Chicago.) Did I understand you to say that you found this disease in various species of snakes? I remember you described one some time ago in a pine snake. Fifteen out of 72 haphazard snakes showing this, in view of the superb vigor and vitality of most of the snakes I have met, to me would indicate it is not a disabling disease, and therefore probably not a true neoplasm.

(Dr. E. B. Krumbhaar, Philadelphia.) Bearing on the question of origin, can Dr. Ratcliffe give us any information on the length of time the snakes have been in captivity? Would that throw any light on this condition — whether it is found more often in captured wild snakes, or in snakes shortly after they have been in the Zoological Gardens, or in snakes that have been there for a longer time and perhaps have become acclimated to an abnormal environment?

(Dr. Ratcliffe.) In reply to Dr. Wells' question about the various species in which the disease has occurred, I have found it in 3 types of rattlesnakes, as well as in non-poisonous reptiles, such as black snakes, King snakes and pine snakes. Very few old world species have been involved.

In answer to Dr. Krumbhaar, the disease has developed chiefly in snakes that have died within a short period after capture, especially in those that seem to be the least adaptable to confinement. Possibly it would be found more commonly in old world species if those that die shortly after capture could be examined.

LYMPH NODE METASTASIS OF SARCOMA (EXCLUDING LYMPHOSARCOMA). Shields Warren, Boston, Mass.

Abstract. Among 237 cases of fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma and osteogenic sarcoma, 18 showed lymph node metastasis. Of the cases with lymph node metastasis, radical dissection of the regional nodes gave more cures and longer survival period in ultimately fatal cases than mere dissection of the involved node.

ANGIORETICULOENDOTHELIOMA (KAPOSI'S DISEASE). A REPORT OF TWO CONSECUTIVE CASES ORIGINATING IN THE HEART. Roger M. Choisser (by invitation) Elizabeth M. Ramsey, Washington, D. C.

Abstract. Kaposi's disease has been known to dermatologists for 76 chronic lesion occurring particularly on the extremities of middle-aged Eastern and Southern European origin. The importance of the standpoint of general medicine lies in the occasional occurrence of lesions in the viscera. Among the 500 cases of Kaposi's disease in the literature to date some 10 per cent show such involvement.

the animals, the denuding effect followed by a dermatitis. If the deficiency is continued long enough the changes produced in the epidermis and corium of the skin simulate, to a remarkable degree, the changes produced by such substances as benzpyrene and methyl cholanthrene on the surface of the skin. The similarity is such as to warrant the speculation that these carcinogenic agents destroy, prevent, or interfere with the same biochemical systems that are interrupted when the vitamin is withheld.

No one can question the tremendous value of Dr. Rous' contributions, but I maintain that an explanation other than his may apply to the results that his superbly ingenious experiments have produced.

(Dr. Rous.) It is possible to explain cancer in any one of a number of ways by simply pushing one's assumptions a little farther than the actual facts warrant, and Dr. Wolbach seems to me to have done that. For example, one can say that cancer is due to a new mutation of somatic type, and if it be objected that no somatic mutations are known which involve a falling away from organization, such as tumors show, the answer can be made, "Yes, but this is a new kind of mutation." In the same way, one can explain cancer as due to an abnormality of mitosis. True, many people have looked at mitoses in carcinomatous tissue and failed to find anything distinctively wrong, but to that the reply is at hand that they haven't looked hard enough or used the right method. Yet again, it can be asserted that cancer results from a permanent change in intrinsic cell activities, and this seems to express Dr. Wolbach's view. To the question, where do examples of this last sort of thing exist in the natural world, the answer can be made that tumors afford them. But this answer cannot suffice: what one must have in the end is facts. And it is an ascertained fact that some tumors are due to viruses. It does not follow that all tumors are referable to such agents, but the fact makes this possibility a good bet for investigation.

(Dr. Joseph Tannenberg, Albany.) I should like to ask whether or not foci of common chronic inflammation such as burns, or granulation tissue produced by infusorial earth, are also foci of predilection when the cancer-producing viruses are administered by way of the blood stream.

(Dr. Rous.) Tar for some reason determines localization of the virus better than anything else we know. If one injures the ear with barium sulphide and injects virus, almost none comes out in the acutely inflamed area, few or no papillomas developing. If the skin is tattooed with sterile needles several hours prior to an intravenous injection, no virus localization takes place at the injured spot, though this is inflamed and edematous. I do not know why that is. Kreyberg and others have shown that tarring the skin renders its vessels extremely abnormal, and probably the virus gets out from them with special ease.

I would like another shot at Dr. Wolbach, or rather at myself. A way to test whether viruses are responsible for the tar tumors would be along the lines he has formulated, namely by a broad study of the growths elicited by a variety of carcinogenic agents, to see whether these evoke the same or different types of tumors. They ought to be of much the same type, irrespective of the carcinogenic agent used, if viruses are their actual cause.

NEOPLASTIC DISEASE OF THE PANCREAS IN REPTILES. Herbert L. Ratcliffe, Philadelphia, Pa.

Abstract. Carcinomatous changes observed in sections of an apparently enlarged pancreas from a pine snake (*Pituophis sayi*) has stimulated systematic microscopic study of this organ in reptiles that have died at the Philadelphia

described in the skin. We agree with Dr. Klemperer that all neoplasms should be designated in histological terms as we did in this instance. Kaposi's disease was added in parenthesis in the title of our paper for identification only.

UNUSUAL NEOPLASMS OF THE SMALL INTESTINE. Samuel A. Goldberg, Newark, N. J.

Abstract. Neoplasms of the small intestine, according to the literature, are rare. With more precise methods of clinical and roentgenological diagnosis an increasing number of such cases is being reported.

During the past 8 years there have been in the Newark Presbyterian Hospital 23,426 surgical cases with 194 neoplasms of the gastro-intestinal tract, of which 9 were in the small intestine. The percentage of tumors of the small intestine is 4.6, which is about the same as the statistics given in the literature. The location and types of these 9 neoplasms were quite different. In this series 3 were in the duodenum, 5 in the jejunum and 1 in the ileum. Examination of the tumors showed 4 adenocarcinomas, 2 leiomyosarcomas, 1 multiple microcystic lymphangioma and 1 hemangioma.

The 3 adenocarcinomas of the duodenum included 1 in the third portion, and 2 in the second portion, 1 accompanied by jaundice, and the other free from jaundice. The 5 in the jejunum included 2 leiomyosarcomas, 1 stenosing annular adenocarcinoma, 1 ulcerating adenocarcinoma and 1 hemangioma.

The tumor in the ileum was a very extensive and unusual lymphangioma characterized by small cysts lined by endothelial and giant cells originating in the submucosa and producing pressure atrophy of the Peyer's patches and the villi, best described by the term *lymphangioma multifforme microcystica*.

Discussion

(Dr. Paul Klemperer, New York City.) I think among the malignant tumors of the small intestine we must recognize the malignant argentaffine tumors. I have seen 10 tumors of this type within the small intestine, some of them multiple, with the typical appearance as in the appendix, 3 of which were resected because of definite clinical symptoms. Of these, 2 showed lymph node metastases. One observed incidentally at autopsy showed a very small metastasis in the liver. It is interesting that the 2 cases in which the lymph nodes were involved reacted very favorably to radiotherapy.

In regard to the tumor which Dr. Goldberg interpreted as a lymphangioma multifforme microcysticum, I wonder if another interpretation might not be possible. The picture reminded me of a disease much more frequent in animals than in man — pneumatosis cystoides of the intestine. I have seen it only in pictures in the intestine, but I have seen the same condition in the bladder, and it has some resemblance to what Dr. Goldberg described, especially the presence of giant cells within the cysts.

(Dr. Theodore J. Curphey, New York City.) Did any of these tumors show regional or distant metastases?

(Dr. Harry C. Schmeisser, Memphis.) I am interested in Dr. Goldberg's presentation for the reason that a few years ago Dr. Robert M. Moore and I, after a brief review of the literature, reported the following 3 unusual cases of benign tumors of the small intestine: (1) cavernous hemangioma of the duodenum, (2) myoma of the ileum, and (3) multiple fibromas of the ileum. The hemangiomas are surprisingly rare; the literature revealed 24 such cases exclu-

nimity of opinion as to whether these lesions represent true metastases or independent primary foci since it has not yet been decisively determined whether the disease is neoplastic or granulomatous.

Two cases recently observed stress the general significance of Kaposi's disease and present certain features not previously described. Both patients were American males in the 3rd decade of life. Both died within 8 weeks from the onset of symptoms diagnosed "influenza." In each the terminal signs were those of "cardiac compression syndrome" and paracentesis revealed bloody fluid in the pericardial cavity. No skin lesions or blood changes occurred in either case. In each, autopsy demonstrated primary involvement of the right auricular wall by an infiltrating tumor mass. In the 1st case the lesion was limited to this location. In the 2nd there was infiltration throughout all of the cardiac musculature and in the diaphragm, liver, lungs and mediastinal lymph nodes.

Microscopically the tumor nodules showed the picture characteristic of Kaposi's disease—namely, proliferation and dilatation of blood and lymphatic vessels, proliferation of connective tissue, areas of hemorrhagic extravasation and pigmentation, infiltration of monocytes, plasma cells and pus cells. Silver impregnation stains (Bielschowsky) showed a massive proliferation of reticulum.

Morcellated portions of fresh tumor tissue and Berkefeld filtrates of such preparations were injected by various routes, subcutaneously, intraperitoneally, intravenously and intracerebrally into a number of experimental animals, rabbits, mice, guinea pigs and canary birds. A localized pyogenic abscess developed in a rabbit at the site of subcutaneous inoculation. Up to the present time (106 and 79 days respectively, since injection), no local or systemic reaction was seen in any case. These negative results are in agreement with those of other workers who have attempted experimental transmission of the disease.

While the age and nationality of the patients and the rapidly fatal course of the disease are unusual features of Kaposi's disease, it is the lack of skin and intestinal lesions and the primary involvement of the heart which are entirely unique. Careful search of the literature fails to reveal any previous report of a similar case.

On the basis of morphology, clinical course and negative results of animal injection, it is concluded that the theory of the granulomatous nature may be ruled out and that the lesion is truly neoplastic. Since the reticuloendothelial system alone can give rise to such diverse elements as compose this tumor and since it is characterized by unbridled proliferation of reticulum and formation of angiomas with hemorrhage and pigmentation, the disease should be classified as an angioreticuloendothelioma.

Discussion

(Dr. Paul Klemperer, New York City.) I wonder if one should apply the term "Kaposi's sarcoma" to tumors that do not originate in the skin. While it is perfectly correct to call these tumors angiomatous, I do not think one should use the term "Kaposi" for a tumor that does not originate in the skin. Kaposi himself saw this tumor exclusively in the skin and later reported metastases only. I do not think one should speak of a tumor that has not originated in the skin, even though it has this angiomatous appearance, as Kaposi's sarcoma. We should use the histological term only.

(Dr. Choisser.) The reason for calling it Kaposi's disease in these particular cases was on account of the identity of the histological structure to what Kaposi

multiple primary malignant tumors is more than a matter of chance. While working during the last year at the Mount Zion Hospital in San Francisco I was able to see in autopsy material from 95 cases 2 cases of double carcinoma. One was a carcinoma of both breasts, both microscopically entirely different types so that a metastasis was completely out of the question. The 2nd case was a carcinoma of the breast and a carcinoma of the stomach, also entirely different types. At the same time among our surgical material a case of double primary carcinoma was observed with carcinoma of the breast removed 10 years ago and a recent myxosarcoma of the ascending colon. In this series no cases were included where a malignant tumor of an organ developed after a carcinoma of the skin because it was felt that skin carcinomas, even though they have the morphological appearance, physiologically do not behave in a malignant fashion. It probably must be assumed that carcinoma is a generalized disease occurring in a predisposed organism. Usually our patients die within a relatively short period after the first carcinoma has been diagnosed and treated. With the improvement in these methods and the longer life expectancy of the patients more and more multiple primary growths will be observed.

(Dr. Shields Warren, Boston.) I want to express my appreciation of Dr. Austin's stressing the need for very rigid criteria in admitting multiple carcinomas. From time to time we run across cases the Residents are very enthusiastic about, but on careful microscopic study they do not turn out to be true multiple tumors.

I might say that in a series of nearly 1000 carcinoma cases which we have been collecting since 1932, Dr. Gates and I have found approximately the same number of multiple tumors that we found up to 1932. I have no doubt Dr. Austin is familiar with the studies of Lund on carcinoma of the mouth where from the clinical angle it was found that the expectation of multiple carcinomas on a chance basis was exceeded 8 to 10 times. Apparently the mouth shares with the large intestine a marked proportion of multiple tumors.

I should like to ask if the age was followed carefully in these cases. The very large number of prostatic cases made me wonder if perhaps a considerable number did not fall in the rather older age groups.

(Dr. Harry C. Schmeisser, Memphis.) I have been much interested in this subject for the last few years because we have quite a number of apparently multiple primary malignant tumors. We found on looking into the literature that they are not particularly rare, and this is borne out by Dr. Austin's tables, but many of the cases may be questioned because the criteria seemed not always reliable. We have insisted on the following minimum criteria: (1) Each lesion must be proved a tumor by its lawless growth. (2) Each tumor must be proved malignant by its invasiveness or metastasis. (3) Each malignant tumor must be proved to be primary by occurring where primary tumors commonly are found, or if located where secondary tumors usually occur, a very careful search must exclude a primary tumor elsewhere. (4) Preferably the primary tumors should be of different specific tissues, as for example, a carcinoma and a sarcoma. (5) Acceptable, the primary tumors should be of different types of the same specific tissue, *i.e.* adenocarcinoma. (6) Least acceptable, the primary tumors should be of the same type of the same specific tissue, as exemplified by 2 squamous cell carcinomas occurring in such locations as to exclude the possibility of one being secondary to the other. (7) Autopsy material is preferable to surgical because it permits of a more complete study. (8) Finally each case must be decided on its merits.

sive of ours. In the literature we found 46 cases of myoma, to which we added 1 weighing 250 gm. Of the 41 cases of fibroma reported in the literature, exclusive of ours, only 2 were multiple. The larger tumor in our case weighed 1140 gm. and the smaller 80 gm.

(Dr. Goldberg.) In reply to Dr. Klemperer's statement it is possible that the lesion in the ileum may be of inflammatory origin because of the presence of giant cells. On the other hand, stasis of lymph or destruction of the fatty tissue in the submucosa might stimulate the production of these giant cells. This specimen has been seen by Dr. Ewing and Dr. Martland and they agree it is a lymphangioma.

In reply to Dr. Curphey's query, of the 10 cases in this series there were 3 myosarcomas, 1 of which showed metastases. One was removed 7 years ago and the patient is still alive. There were 5 adenocarcinomas, 3 of which showed metastases. The annular carcinoma of the jejunum was removed a little more than 2 years ago and the patient is still alive. The other 2 cases were benign neoplasms.

MULTIPLE PRIMARY MALIGNANT TUMORS. Richard S. Austin, Cincinnati, Ohio.

Abstract. In the performance of 8124 autopsies malignant tumors were encountered in 887 cases, of which 24 presented more than one type of primary carcinoma. This is a frequency of 2.7 per cent, which is higher than can be explained on the basis of chance. Cases of carcinosarcoma have been included, but not cases of malignant tumors of a single histological type but multicentric in origin. Except for 2 of the 24 cases of multiple carcinoma, at least one of the types of carcinoma in each case had its primary site in some portion of either the digestive or urogenital systems.

Discussion

(Dr. H. Gideon Wells, Chicago.) One point I think in favor of the view that these figures that you give are more than chance and indicate something in the way of a systemic susceptibility is the experience of all people who are producing lines of animals with a high incidence of tumor — namely that in animals of long ancestry of high tumor incidence we get a surprising number of multiple tumors. Dr. Slye has had mice with 6 or 7 different tumors in the same mouse, and one animal was made up of about 80 per cent tumor tissue, indicating that these mice have a peculiarly high susceptibility to carcinoma. Mice often have 6 or more tumors of the breast at the same time.

(Dr. C. V. Weller, Ann Arbor.) Dr. Austin made the statement that the incidence of multiple tumors is very much higher than chance alone can explain. I sympathize with that view. I am glad to hear him express that opinion; it is in line with my own thought, but I would like to know how he arrived at that conclusion — by what statistical method. Dr. J. C. Bugher worked in my department for 2 years on a very complex and difficult mathematical analysis of this question, in which he attempted to derive the equations for the curves of tumor incidence. If you will read his paper carefully you will find that even after this detailed mathematical study the implication is not too strong that he proved that these tumors occur more frequently than chance alone can explain. I should like to know whether Dr. Austin arrived at his conclusions by the mathematical method.

(Dr. Cronheim, Albany.) I am personally convinced that the occurrence of

possible that among the cancer susceptibles there are varying degrees of susceptibility, so that in some cases primary carcinoma is not confined to the same organ or system but involves others.

Some of the discussion does not need any comment, but I should like to speak of some of the questions which Dr. Warren raised. I am acquainted with Dr. Lund's work. He included some tumors of the same type as "multiple tumors," but I do think his publication on that basis is quite significant. As far as the age of the patients goes, the general distribution is about the same as that of cancer patients. Five of the prostatic tumors were in men above 70 years of age.

Dr. Schmeisser said he thought we should be even stricter in our criteria. I thought I was leaning over backwards. I did count what I call "double carcinoma" cases; they are different types in different sites, ruling out the metastatic situation. As far as the respiratory system is concerned there were no primary carcinomas of the lung in the series. They were either fibrosarcoma or endothelioma coincident with carcinoma elsewhere. There was no question of metastasis to the lung.

In regard to Dr. Hastings' remarks, if we did include benign tumors we would certainly have a very high total of cases of multiple tumors.

IDIOPATHIC CARDIAC HYPERTROPHY WITH COR PULMONALE. Norbert Enzer, Milwaukee, Wis.

Abstract. A white male 29 years of age died after 3 weeks of progressive cardiac decompensation of abrupt onset. At postmortem examination a massive hypertrophy of the heart was discovered for which no adequate explanation was apparent. The right chambers were enormously distended and their walls greatly thickened.

A review of the literature and a discussion of the possible explanations for this hypertrophy are offered.

Discussion

(Dr. Maude E. Abbott, Montreal.) Were the tissues stained for glycogen?

(Dr. Benjamin J. Clawson, Minneapolis.) I have been interested in this group of hearts, not only in children and young people but in older people. We have found quite a number of cases with cardiac hypertrophy, dilatation and congestive failure where the clinicians over quite a long period of time, for several years, have repeatedly taken blood pressures and have found them not to be elevated. Whether these are the same as the case reported by Dr. Enzer I do not know, but there does seem to be a group of adults who do have idiopathic cardiac hypertrophy.

(Dr. Alfred Plaut, New York City.) What was the condition of the pre-capillary small vessels in the lung?

(Dr. Enzer.) In reply to Dr. Abbott, the postmortem examination was done 4 months after death. We tried to stain the tissues for glycogen. None was found, but I am not certain that it was a proper specimen for the determination of glycogen.

The clinical history in the case indicates this patient had a normal blood pressure. The only defect in the examination was the absence of electrocardiographic tracings, of which none were obtained.

In reply to Dr. Plaut, we were particularly interested in the lungs and made large serial sections of numerous areas, studying the vessels and elastic tissues, and found them entirely normal as compared with individuals of the same age.

(Dr. Esmond R. Long, Philadelphia.) It would seem to be about as bad an error to omit cases through excessively rigid criteria in interpreting a double primary carcinoma as to make a mistake in the opposite direction.

(Dr. Joseph Tannenberg, Albany.) According to my experience in a large German pathological institution that had about 1800 autopsies a year, I should think that multiple malignant tumors are still more frequent than is expressed by the statistics reported by Dr. Austin. I think so because, from this institution and others of similar size, single instances of multiple carcinomas were not reported in the literature, and therefore would be absent from the collective statistics that comprise the cases reported in the literature.

As to the question whether or not 2 carcinomas of the same case should be counted as multiple malignant tumors, I can see no reason for not counting them when they differ enough in their location and histology, as, for example, a squamous cell carcinoma in the esophagus and an adenocarcinoma in the rectum.

(Dr. N. Chandler Foot, New York City.) One thing that should be brought out in connection with this is the importance of studying the patients during life as well as on the autopsy table. I think many multiple tumors may be missed in that way. I have a definite case in mind. We had a patient who came into the Hospital in 1934 with a malignant tumor of the carotid body, a very unusual type of carotid body tumor. In 2 years she was back and had a perfectly typical adenocarcinoma of the colon which necessitated a subtotal resection. This second tumor was totally unrelated to the malignant tumor of the carotid body. Had that woman gone to some other hospital in some other city and later come to us, the chances are we should have known nothing about the first tumor at all. This patient is still alive and well.

(Dr. Willard S. Hastings, Philadelphia.) A question which interests me very much in this connection regards the significance of including benign tumors in such a study as this. I do not know what has been done in this way. Since the borderline between benign and malignant is often so indefinite, multiple neoplasms of all types might be expected to follow the same general rule as the malignant ones alone, and be somewhat less subject to the uncertainty coming from metastasis. The problem may perhaps be somewhat less interesting, however. I think most of you will agree that the occurrence of leiomyomas of the uterus in an autopsy series that is made up largely of malignant tumors of other parts will be very much according to the laws of chance. The same is true of the pigmented nevi, if we consider them neoplasms. The autopsy material in our institution is largely made up of malignant cases, and I think, without ever having counted them, that these two examples of benign tumors occur in much the same number as in the general population. Of course instances of multiple tumors are numerous if we include the benign ones.

(Dr. Austin.) In reply to Dr. Weller, I am acquainted with the article to which he refers in which the preponderance of the evidence was that the tumors occurred more frequently than would be due to chance, but the author could not prove it. Perhaps I overstated the case; a number of articles have attempted statistical analysis, comparing the population and cancer incidence, and multiple cancers seem to occur more often than chance would indicate. It is probable that this is significant, although I do not think it has been mathematically proved.

As far as Dr. Wells' comments are concerned, we know that a fraction of the population is highly susceptible to cancer. If there are differences in degree or kind between the non-cancer susceptibles and the cancer susceptibles, it is

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where pathologists cannot agree — the one sometimes referred to as blast cell (stem cell) leukemia.

(Dr. J. Earle Ash, Washington.) I should like to ask where Dr. Moon places the lipoid-reticuloendothelial disease and Hodgkin's disease.

(Dr. Herbert Fox, Philadelphia.) I would be glad if a system of such simplicity could be agreed on and accepted by pathologists, but I wonder to what degree it is very helpful, and this is no reflection on the speaker, to the pathologist who has to examine biopsy material, and whether he can classify material that is handed to him and sometimes cut out badly by the surgeon, and apply these AB's. I quite agree with the whole effort. I think there are some cases in which it would not entirely hold, but if the pathologist be consulted on the selection of material on which to make a biopsy, and can advise in selecting a suitable gland, not the oldest or the youngest, away from the drainage line, and if suitable stains are applied, perhaps we would come closer to adopting what Dr. Moon has suggested.

(Dr. Charles H. Bunting, Madison.) We always appreciate an attempt to simplify these various lymph gland- marrow-leukemic pictures, but I think, with due respect to Dr. Moon, that we have something here which Nature does not classify. There is one point I have always made to students, and that is that in the various leukemic states we have an involvement of both marrow and lymphoid tissue. In the so-called myeloid leukemia one quite constantly finds a myeloid transformation of lymphoid tissue. On the other hand, I have never seen a lymphoid leukemia without marrow involvement, so I think that while primarily it is a good idea to try to classify these things, at the same time we must remember that all parts of the hematopoietic system are involved in all of these leukemic and aleukemic states.

(Dr. Norbert Enzer, Milwaukee.) The late Dr. Jaffé made a very pertinent remark with respect to the classification of leukemia. When he was presented with a very laboriously worked out scheme for the classification of leukemia he remarked: "That is splendid — now let us see if we can make leukemia behave that way," and I think that those of us who see occasional cases of leukemia would be hard put to it, as Dr. Fox has pointed out, to place these cases in the scheme of classification which Dr. Moon has presented. It might be more serviceable for the classification of completely worked out postmortem material. Dr. Herrick made a remark last night which could well be applied to this situation with respect to the teaching of students of so complex a subject as leukemia. Perhaps we should not close the doors of their minds to the complexity of the subject.

(Dr. Moon.) I will say in advance I am very grateful for the interest this has excited. If it has done no more than that it has accomplished in large measure its purpose.

A question was asked regarding those cases in which the type of cell is not apparent. It is not possible to classify either leukemic or other neoplastic conditions if one cannot recognize the type of cell concerned. This scheme of classification would not be applicable to such cases. There must be the determination of the type of cell; also the determination of whether the case has or has not leukemic blood, and the presence or absence of a local tumor growth. Given those three items (and without them one cannot classify anything) one can at least arrive at a designation which will tell the other fellow what this is.

I do not believe this classification will apply to Hodgkin's disease. I am not at all sure what Hodgkin's disease is. Whether it is related to the leukemic con-

A CLASSIFICATION OF LEUKEMIC AND ALLIED CONDITIONS. Virgil H. Moon, Philadelphia, Pa.

Abstract. Years ago Sternberg proposed to differentiate these conditions according to the type of cells, the presence or absence of local invasive growth and of leukemic blood. Later MacCallum used the same criteria in an amplification of Sternberg's classification. This same scheme expanded to include reticuloendothelial cells provides a simple and practical classification for this entire group of disorders.

Three main divisions are determined by the three types of cells involved: lymphoid, myeloid and monocytic (reticuloendothelial). These are further subdivided according to the presence or absence of leukemic blood and of local invasive neoplastic growths.

Let the presence of leukemic blood be represented by the symbol +A and its absence by -A. Also let the presence of malignant neoplastic growth be designated by +B and its absence by -B. The possible combinations of these features are as follows:

I. Lymphoid hyperplasia

- +A -B Lymphoid leukosis (or lymphoid leukemia), acute or chronic
- A -B Aleukemic lymphadenosis (pseudoleukemia)
- +A +B Leukosarcoma
- A +B (1) Primary neoplasm of lymphoid tissue: lymphosarcoma
- (2) Primary neoplasm of bone marrow: lymphoid or plasma cell myeloma.

II. Myeloid hyperplasia

- +A -B Myeloid leukosis (or leukemia), acute or chronic
- A -B Aleukemic myelosis
- +A +B Leukomyelosarcoma
- A +B (1) Myeloid tumor not originating in bone marrow: myelosarcoma
- (2) Myeloid tumor of bone marrow: myeloid myeloma

III. Reticuloendothelial hyperplasia

- +A -B Monocytic leukosis (or leukemic retiotheliosis)
- A -B Aleukemic retiotheliosis (giant lymph follicle hyperplasia?)
- +A +B Leukoretiothelioma (or leukoretosarcoma)
- A +B (1) Neoplasm of retiothelial tissue: retiothelioma (or retosarcoma)
- (2) Retiothelial neoplasm of bone: Ewing's tumor (?)

This scheme provides for a systematic grouping based on morphological features of the leukemic and related conditions. It simplifies discussions and greatly facilitates the didactic presentation of this subject to students. Obviously this system will not classify conditions in which it is not possible to determine the type of cell, the presence or absence of local neoplasia and of leukemic blood. But no other classification is possible when these items are indeterminate.

Discussion

(Dr. Harold Gordon, Louisville.) I should like to ask Dr. Moon where he would place those leukemias where the type of cell is not readily determined, or

Discussion

(Dr. Virgil H. Moon, Philadelphia.) I am particularly interested in this presentation because of the interest we had in this subject 10 years ago. In that series in which we compared the splenic weight of 1000 whites above 18 years of age with that of 1000 negroes of similar age, there was a very significant difference. Many of the points which Dr. Krumbhaar noted were evident also in our survey of 2000 cases. Our patients all died of disease. But in every instance we eliminated all spleens enlarged by disease, and also those above 350 gm. in weight because they could confuse the ratio of normal splenic weights — the item for which the survey was made. In that series it was shown that the splenic weight was greater in the male than in the female, and that it declined with age. It was highest at the period of 18 years, the lowest age in our series. I wish to ask Dr. Krumbhaar approximately the ratio which was established in his series between the weight of the white and negro spleens respectively. I may say that in our series it appeared that the spleens of negroes were approximately two-thirds the size of those of whites of the corresponding age, sex and body weight.

(Dr. Carl V. Weller, Ann Arbor.) As far as I can tell from the presentation, Dr. Krumbhaar's results are comparable in every respect with those that Ahronheim obtained with us in a group of 1000 spleens. I should like to ask in respect to the group of accidental deaths whether it might not be that the error resulting from hemorrhage is not even more than offset by the fact that those who possess the lymphatic constitution, and therefore have large spleens, will be represented in that group in a larger proportion than in the general population. This might be true in deaths from drowning, for instance. I am convinced that an individual with an excess of lymphatic tissue will die under degrees of submersion from which another individual might be resuscitated. In that respect you may be obtaining a balancing correction between the two errors.

Another point which is of no great importance, except from the standpoint of statistical analysis, is the alternation in the curve of the 5 year age periods. I noted in the curve that the hemidecades ending in 5 gave results several grams higher than those ending in 0. Have you an explanation for this? The only one that occurs to me is that this must, in some way, be related to the well known error of approximate answers in respect to age.

(Dr. Paul Klempner, New York City.) I want to ask whether the high weight of the spleen in the 16 to 20 year period is due to the larger amount of lymphoid tissue.

(Dr. Emmerich von Haam, New Orleans.) I should like to ask if there was any estimation of the difference between the contracted and the dilated spleens. In my experience with splenectomized animals I found that the difference in contracted and dilated spleens can be as high as 30 per cent of the weight of the organ. I should like to ask if such a possible factor was taken into consideration in the determination of the normal weight.

(Dr. Krumbhaar.) In reply to Dr. Moon, I am confident that the spleens of blacks are notably lighter than those of whites. While we did not make quantitative comparisons, I would say that their weight was something like two-thirds, or possibly three-fourths that of whites. I might also say that while Dr. Moon got his figures from Blackley for his article published in 1928, and many of ours were from the same source, yet all of ours were from cases after 1928, so that there was no overlap.

Dr. Weller spoke of Ahronheim's paper. We are of course conversant with it, and I may say that we got essentially the same results that he did, though work-

ditions has not been established, but most pathologists are able to recognize Hodgkin's disease without confusing it with the lymphoid hyperplasias. There are difficulties on that point, and if one cannot determine them one cannot classify them.

I agree with Dr. Fox that the selection of biopsy material is important. Practically the only satisfaction we are going to get out of this group of conditions is our ability to put them into a pigeonhole. I believe we have formulated a scheme of classification which will aid in doing that.

I agree with Dr. Bunting that frequently both lymphoid tissue and bone marrow are involved in these conditions, but this does not contradict the fact that the predominating cell in the hyperplasia is myeloid or lymphoid correspondingly. Sometimes two types of cells are participating in the hyperplasia. One advantage of this scheme is that it provides a logical designation for such cases. One can easily supply the combined designation, as suggested in the last slide. It is because of its adaptability to such situations that I have found the scheme quite useful.

THE EFFECT OF AGE, COLOR AND SEX ON THE WEIGHT OF THE HUMAN SPLEEN.

E. B. Krumbhaar and (by invitation) Stuart W. Lippincott, Philadelphia, Pa.

Abstract. It is surprising but true that we do not yet have acceptable values for the weight of the human spleen at different age periods, as influenced by color and sex. We have therefore attempted to secure more adequate figures, in two series, as follows: (a) an analysis of 2000 routine autopsy cases (omitting all those in which any considerable splenic lesions were found); and (b) an analysis of 2000 cases of violent death (where except for the shrinking effects of shock and hemorrhage and the swelling effect of a few cases of minor sepsis, the spleen was supposedly normal). In addition to a comparison of body weights, the data were further analyzed according to sex and color of the individuals, and according to the chief cause of death and the histological appearance of the spleen.

Curves of the mean weights of the spleen for the several age groups were made for each series and were found to be strikingly parallel. As spleens in the first series were thought to be heavier than the hypothetical normal and those in the second series lighter, for the reasons given above, a curve for the combined 4000 cases was constructed. This, in our opinion, comes nearest to the true average "normal" values of weights of human spleens at different ages. The spleen appears to reach its greatest weight in the 16 to 20 year group and to maintain a slightly lower weight through adult life to the age of 65 years, after which it loses in weight. In relation to the body weight the spleen is heaviest in the first age group (ratio of 0.0042), the ratio decreasing slightly to the age of 30. From there on the ratio of weight of spleen to body weight remains constant, or nearly so. Analysis according to sex and color shows that males have heavier spleens than females after the age of 15 years, and that negroes have lighter spleens than whites. Color appears to be a stronger factor than sex, as the spleens are heavier in the white females than in the male blacks. Analysis of the causes of death and of the histological appearances of the spleens brings out two points: (1) respiratory diseases are most frequent in the two age groups that have the heaviest spleens, and as such diseases tend to produce heavier spleens, they constitute a factor in the heavy weight in these groups; and (2), arteriosclerosis becomes an important factor in the older cases, and thus may be correlated with the lighter spleens found in the older age groups.

fifth and sixth decades. The results indicate quite definitely that (1) the bacilli responsible for the lesions comprising the primary complex of pulmonary tuberculosis eventually become non-viable and (2) that the lesions in most instances represent a process that has essentially healed.

Discussion

(Dr. Max Pinner, Ithaca.) I am much interested in this study. Anyone who has searched the literature for the bacteriological behavior of the primary complex knows that the available data are confusing because the differentiation between true primary complexes and other encapsulated or calcified lesions is rarely stressed. I should like to hear more from Dr. Feldman of how definitely the differential diagnostic criteria for true primary foci have been applied in his study. I believe that some of the slides shown are rather characteristic of reinfection foci.

In regard to the final and important conclusion as to the possibility of endogenous reinfection, it is important to point out that it is not an infrequent finding to see definitely active tuberculous lesions in the lymph drainage between hilum and venous angle in the presence of completely calcified or ossified primary complexes. In other words, lymphatic spread from the lymphoglandular component of the primary complex may occur before anatomical and bacteriological healing of the latter is complete. From such satellite foci of the primary complex endogenous reinfection may occur in the presence of completely healed primary complexes.

(Dr. Norbert Enzer, Milwaukee.) What method did you use for the demonstration of silica in these preparations? From the lantern slides I would not have suspected these to be silicotic lesions.

(Dr. David Perla, New York City.) I think this work is very important and it completely confirms the earlier work of Opie, who showed a number of years ago that when the primary complex was encapsulated with a fibrous capsule and was caseous, it rarely contained viable bacilli. I happened to be with him when he was doing this work and I was very much impressed with the uniformity of the negative results obtained by culture or guinea pig inoculation of such primary lesions. Not infrequently in normal lung tissue about these lesions could viable bacilli be obtained, and Dr. Opie made an effort to control the contamination of the foci with the normal lung tissue, as Dr. Feldman did. I think to some extent Dr. Feldman is justified in the conclusion that it is difficult to decide whether a secondary infection can arise from such a primary focus when no viable tubercle bacilli can be demonstrated in such primary complexes.

(Dr. Esmond R. Long, Philadelphia.) This paper was interesting to me too in connection with an observation that is becoming increasingly common among epidemiologists engaged in the study of tuberculosis, *e.g.* a negative tuberculin reaction in the presence of obvious calcified foci of tuberculosis. It used to be thought that the tuberculin was not potent enough to detect the allergy left from these tubercles, but the probability is that these lesions are truly obsolete and that the tubercle bacilli once present are dead. Correspondingly when the tuberculin reaction is positive in the presence of old calcified foci, such as Dr. Feldman showed, it is not always positive because of the calcified foci. I have come to believe it is positive much more commonly because of some small subsequent reinfection.

(Dr. Feldman.) Dr. Pinner's remarks are pertinent, but he has set up a very difficult question for me to answer. In this instance it was difficult because I

ing from a slightly different point of view. There has been one very good paper on the lymphatic content of the spleen by Hellman of Stockholm, and he paid considerable attention to cases of lymphatism. We did not; the nature of our series prevented us from doing so. I cannot therefore say anything definite about it in our series, but I would not have thought it played a very important rôle. Even in the cases where shock and hemorrhage were listed, which theoretically ought to reduce the weight of the spleen, we found in some groups that these spleens averaged more than those of the rest for the same age period. In general, however, they were lower, but as shock and hemorrhage were undoubtedly mentioned much less often than they actually occurred, it was impossible to evaluate this feature with any accuracy.

We did notice the alternating ups and downs in the 5 year periods, but can offer no explanation other than the chance variation to be found even in such a large series.

In reply to Dr. Klemperer, it is true that the percentage of lymphoid tissue tended to be higher in the ages where the spleen was heaviest. To that extent it was a factor in the increased weight. In view of the relatively small differences that we get in the lymphoid percentages, however, I think it will probably prove to be a minor factor.

In regard to Dr. von Haam's remarks, we could make no attempt to control the difference between the contracted and the dilated spleens. Of course we are familiar with the work of Barcroft, who first demonstrated quantitatively the reservoir capacity of the spleen in several species of animals. He found that the cat, with the most muscular spleen, could squeeze out more blood than the other species he studied. As I recall, the cat could expel about 33 per cent of the total spleen volume, while figures for other species were as low as 20 per cent. Possibly the average human spleen has the ability to squeeze out some 25 per cent. After maximum expulsion there is, of course, still a lot of blood left behind. If you remove a spleen at autopsy, even several hours after death, you know how much blood drips out, and if you perfuse it, a lot more is expelled, so that the amount of blood in the normal human spleen during life is considerable. It has been estimated at about 50 to 60 cc., but the exact figure will be impossible to get for a long time, if ever, because normal human spleens are not removed from the body sufficiently frequently at operation for study.

THE RESIDUAL INFECTIVITY OF THE PRIMARY COMPLEX OF TUBERCULOSIS.

William H. Feldman and (by invitation) A. H. Baggenstoss, Rochester, Minn.

Abstract. A study was made to determine the presence of viable virulent bacilli of tuberculosis in chronic tuberculous lesions of the lungs and contiguous lymph nodes of human beings dying of causes other than tuberculosis. The lesions were emulsified and material from the respective cases was used to inoculate culture mediums and to inject guinea pigs. Material from a total of 68 cases was utilized and negative results were obtained in all except 1 case. Tissues from the lesions of most of the cases were studied microscopically and evidence of activity was apparent in a considerable number. In lieu of demonstrable viable tubercle bacilli that could account for the persistence of the activity on the part of the morbid process, a possible explanation was found in the presence of silicon within most of the lesions. The age distribution of the individuals studied ranged from 7 to 90 years with approximately 70 per cent in the fourth,

was not able to observe it by gross examination, when I examined it microscopically I could see the lesions had been there, so that the tendency, even with the clearing up of the tuberculosis in the right lung, was toward the healing of the lesion in the left lung.

VARIATIONS OF PULMONARY LESIONS IN PRIMARY AND POSTPRIMARY HUMAN TUBERCULOSIS. Kornel L. Terplan, Buffalo, N. Y.

Abstract. The anatomical lesions in cases of progressive tuberculosis in children include many variations. A combination of marked bronchogenic spread from an unusually large caseated Ghon focus, with extensive lymph node changes and hematogenous miliary tuberculosis, is one example. Another variation showed only extensive bronchogenic spread from a huge primary pneumonic focus, with considerable cavitation, minimal hematogenous spread and minute lesions in the lymph nodes draining the primary cavity.

About 45 per cent of the cases examined may be considered as examples of the classical picture of primary tuberculosis in children, where there is a cheesy complex, with lymph node changes extending into one or both venous angles and overwhelming miliary tuberculosis.

Some instances point to exogenous reinfection — the presence of a calcified complex in one lung, and in the other lung a second and more recent complex. This was especially impressive in a child 5 years of age, where the second infection had led to fatal hematogenous dissemination.

Among these cases in which the finding of tuberculosis was incidental, there are some foci in healing or healed stages entirely without lymph node changes. Complete serial sections of the lymph nodes draining the foci were carefully studied and no spread to these structures could be demonstrated. Similarly, a completely ossified focus in the process of resorption, surrounded by a few bony fragments, with completely negative lymph nodes, both subpleural and those of the tracheobronchial tree, points to the possibility that the first tuberculous infection may be restricted to the lung tissue.

In 1 case of a child, 10 years of age, cheesy fibrous foci (about 9) were found in different lobes with no lymph node changes whatsoever and no evidences of hematogenous spread.

Complete lobar collapse from a stony focus occluding the main bronchus, and recent tuberculous meningitis originating from endogenous lymphoglandular reinfection within the almost completely calcified regional lymph node in the venous angle, are rare variations. Endogenous lymphoglandular exacerbation within a calcified complex, with activation of tuberculosis in the lymph nodes regional to the focus, leading to tuberculous meningitis but without miliary dissemination, was seen in 3 cases. Finally, 1 or more stony foci may be found obstructing bronchi of second or third order with collapse induration of the lung tissue tributary to these bronchi, thus presenting the end stage of the so-called epituberculosis.

Variations in adults are also encountered rather frequently: in several cases Ghon foci, still active or healed, either single or multiple, in different lobes but having the same histological structure, were found to be completely without lymph node changes. In other cases single foci in different lobes were found, again the nodes showing no changes, but the foci of different structural age. In several instances there were findings pointing to bronchogenic extension, with formation of isolated foci, both single and multiple infections, in different lobes,

had to rely on others to do the autopsies, and we only received the material which was used for the subsequent studies. I emphasize again that only material in which tuberculosis was not a cause of death was used, and in the majority of cases we were dealing with what seemed to be old Ghon tubercles. I am sorry I cannot give you more specific information. We decided that since we could not demonstrate tubercle bacilli by what are considered to be rather sensitive tests, guinea pig inoculation or culture, these chronic, apparently healed lesions are not likely to be a factor in so far as endogenous infections are concerned.

Dr. Enzer asked as to the method of detecting silica. We only assumed this was silica. We used the polarizing microscope. We realize the shortcomings of the method but the material looked like silica.

THE EFFECT OF THE PRIMARY PULMONARY FOCUS UPON THE PROGRESS OF
EXPERIMENTAL PULMONARY TUBERCULOSIS. B. J. Clawson, Minneapolis,
Minn.

Abstract. A primary focus of tuberculosis was produced in the left lungs of rabbits by injecting 2 mg. of BCG directly through the pleura into the lower lobe. These animals with a few exceptions became allergic (+ to +++) as shown by positive Mantoux tests 3 weeks after the BCG injection. A circumscribed tubercle developed.

The allergic animals and normal animals were then injected with 0.01 mg. of a virulent bovine strain into the right lung 40 days after the BCG injection into the left lung. There were 32 normal animals and 38 animals with the primary focus. Both kinds were killed at intervals of from 1 to 110 days and the amount of tuberculosis in the right lungs compared in the two groups.

For the first 7 days the rate of development was greater in the allergic animals. Then the development was retarded or completely stopped. From the 7th day the rate increased in the normal animals up to 28 days and beyond, when all normal animals had extensive tuberculosis. In all but 1 of the animals with the primary focus there was no tuberculosis or a marked retardation up to 110 days.

A primary pulmonary focus increases the rate of development of a secondary virulent infection for a few days, and then retards or completely prevents the development of tuberculosis.

Discussion

(Dr. Esmond R. Long, Philadelphia.) It is interesting to note that in spite of the good protection ordinarily afforded by the immunization, there are always 1 or 2 animals that do not seem favorably affected. Dr. Clawson has 1 vaccinated animal with very extensive tuberculosis which seemed to differ from the 15 to 20 that had been well protected. This seems to conform with certain of Dr. Lurie's observations on native resistance.

(Dr. Norbert Enzer, Milwaukee.) I should like to ask whether in the primary focus of the left lung there were any satellite foci or secondary spread in that lung during the presence of the spread of tuberculosis in the opposite lung.

(Dr. Clawson.) In regard to Dr. Long's question of just why this animal developed a four plus reaction, I do not know. It always happens. I can never get 100 per cent protection.

In regard to the spread of the primary infection in the left lung, if there is a flare-up of the infection of the right lung — no, there is none. In some of the cases it became small, and in a few cases it disappeared, and in others where I

the cells were viable as demonstrated when growth occurred after subculturing a colony. The chromosomes in many cells were increased in numbers much above the diploid, a change produced perhaps by dispersion of longitudinally split chromosomes, such as might form during the normal prophase or metaphase. Clumping of chromosomes into dense groups was observed soon after the dispersion. As early as 9 hours after the introduction of the colchicine some of the cells were seen to contain many small nuclei ("micronuclei" of Brues), probably derived from swelling of the clumped masses of chromatin, but many of the cells underwent necrosis with extrusion of pyknotic debris. Concentrations as low as 1:128,000,000 produced minimal effects, but only after a longer interval of time. Studies of the growth rates showed inhibition of growth of the cultures even with such concentrations of colchicine as produced a minimal morphological effect.

Ethylcarbylamine, whose capacity for inhibiting the Pasteur effect was demonstrated by Warburg, was found to produce changes in mitosis identical in character and sequence with those effected by colchicine. The range of concentrations was smaller, 1/2500 to about 1/15,000. The rapidity of transformations was greater and the accumulation of arrested mitoses was less. By 9½ hours at 1/6250 very few cells with dispersed chromosomes were seen. Most of them showed pyknotic masses of chromatin, micronuclei or resting nuclei. At 24 to 30 hours extremely few cells were found in mitosis, although such mitoses as were present were typical or contracted. Some colonies showed no mitosis. Despite this the cells were proved to be viable by subculture. Subcultures were successful also at earlier stages of action of a concentration of 1/6250. The growth rate was diminished at this concentration from about the 1st to the 9th hour, but approached that of the control cultures again during a subsequent interval of time.

DIRECT PATHOLOGICAL EVIDENCE OF CIRCULATORY DISTURBANCE IN THE BRAIN. STUDIES WITH THE BENZIDINE STAIN. Leo Alexander and (by invitation) Tracy J. Putnam, Boston, Mass.

Abstract. The benzidine stain is the best available method for the study of pathological alterations of the vascular pattern of the human central nervous system. It is also valuable for the study of normal vascularity, especially in regard to the size of normal vessels.

The benzidine stain (Lepehne-Pickworth) demonstrates the vascular pattern by selective staining of the red blood cells contained in the vessels. The method consists essentially of applying the benzidine test for hemoglobin (peroxydase reaction) to thick frozen sections of 200 to 300 μ thickness. In addition to the red blood cells, the polymorphonuclear leukocytes are stained also, but no other tissue elements.

This study is based on 62 pathological cases and a number of normal controls.

The pathological alterations of the cerebral vascular pattern that are demonstrable with the benzidine stain are mainly the following, which may occur either independently or together in the group of pathological conditions we had the opportunity of studying. These main types of alterations are: (A) *Simple vascular dilatation, generalized or local.* (B) *Vascular dilatation with bead-like deformity of contour ("beading")* of the vessel or vessels involved. (C) *Hemorrhage.* The finding of changes of the above 3 types (A, B, C) can be accepted as representing the true condition at the time of death. These changes

sometimes in subapical position. In later stages such foci may show the same histological structure as the primary focus, including complete ossification.

In addition to the group of cases already reported at the Boston meeting (1936), several more cases of complexes of different ages have been studied, especially in young individuals between the ages of 20 and 30 years. In one of these a recent hematogenous dissemination had occurred in connection with the cheesy complex of the postprimary infection, while the primary complex was completely calcified. That apical and subapical focal lesions occur by direct bronchogenic spread from the primary focus was seen in several instances, in many of which the histological structure was similar to that in the primary focus. The complex changes in the lymph nodes regional to the focus were not entirely healed. However, no hematogenous spread was seen in any organ.

We feel that those incidental findings in postmortem examination of such individuals as do not die from tuberculosis present a most useful material for pathogenetic studies of this disease, in that they may prevent us from being too dogmatic in the teaching of the types and development of primary and post-primary tuberculosis. Indeed the anatomical course of primary or postprimary silent tuberculosis in the average healthy individual, where there appear to be no clinical symptoms, is unknown. It seems, however, from our postmortem material, that the anatomical lesions show many individual variations, and this applies as well to the lesions of cases recognizable clinically. For scientific purposes, then, deductions from tuberculin tests, which were strikingly incorrect in 2 cases in our own experience, and from X-ray interpretations, the difficulties of which are well known, are not sufficiently adequate. As in the days of Parrot and Kuss, only the most thorough anatomical examination of the human cadaver, as so classically practiced in the service of Dr. Ghon, linked with systematic X-ray photographs of the lungs and tracheobronchial tree, together with the most detailed histological investigation of all the lesions encountered, may still lead to a more exact and complete knowledge of the problem of tuberculosis.

THE ACTION OF COLCHICINE AND OF ETHYLCARBYLAMINE UPON A MOUSE
BREAST CARCINOMA IN VITRO. Averill A. Liebow (by invitation) and
Robert Tennant, New Haven, Conn.

Abstract. In a study of substances influencing the growth and morphology of cells in tissue culture the action of colchicine and of ethylcarbylamine were found of particular interest. The effects of these substances on a transplantable mouse breast carcinoma and also on normal embryonic tissues of the mouse, rat and humans were essentially similar. The tissues were grown in Carrel flasks in a medium of washed chicken plasma clot with a supernatant of rat serum diluted with two parts of Tyrode's solution, except in the case of the human tissue where human placental serum was substituted for rat serum.

Within 30 minutes after the introduction of colchicine in a concentration of 1 to 8 million all of the mitotic figures were comprised of shortened thick chromosomes arranged in a roughly spherical mass near the center of the cell. Within an hour the chromosomes in some cells had become much shorter and had become widely dispersed throughout the cytoplasm as described by Ludford. By the end of 2 hours all of the mitoses showed these changes and no anaphases or telophases were seen. There was a large increase in the number of mitoses which was maximal at about 9 hours. Despite such dispersion of chromosomes

cannot be imitated by postmortem changes. In the vast majority of cases they are a valid part of the ante mortem changes, being surprisingly slightly, if at all, modified by agonal phenomena. The distinction between true bead-like dilatations of vessels and small sheath hemorrhages or dissecting aneurysms is not always easily defined in benzidine stained preparations. Neighboring sections stained with hematoxylin-eosin or Masson's trichrome stain should always be examined. (D) *Capillary anemia, with or without hyperemia of larger vessels, sometimes mixed with hyperemic zones either in the center or at the periphery of the lesions.* Care should be taken in interpreting the changes of this type not to confuse them with the artificial appearance of anemia that may be produced in regions of the brain that may have been compressed by handling at autopsy, by postmortem alterations of distribution of blood, or in regions that may have undergone shrinkage when first exposed to the fixative. The changes of this type may be accepted as valid only if encountered in the lesions of brains that show a uniformly normal blood supply throughout the neighboring normal areas. (E) *Destruction of vessels of a certain category, notably involving those of first and second order.* (F) *Diminution of vascular caliber.* Neighboring sections stained according to the usual methods (hematoxylin-eosin, Masson's trichrome stain, and so on), which should always be examined in conjunction with any benzidine stained preparations, will have to be confirmatory in order to establish the validity of any observation of a change in these types (E and F).

These pathological changes of the cerebral vascular pattern are found in the following conditions: (A) *Generalized simple vascular dilatation* occurs in generalized anoxemia or hypoxemia, e.g. with respiratory embarrassment. Localized simple vascular dilatation occurs: (1) in acute local anoxia, e.g. with arterial occlusion; (2) in compression of meningeal veins, e.g. from non-specific meningo-fibrosis in tuberculosis; (3) in the diseased posterolateral areas of the spinal cord in combined system disease in pernicious anemia; (4) in extensive thrombosis of intracerebral vessels, notably in the white matter, e.g. in arsphenamine poisoning; and (5) in poliomyelitis acuta anterior. (B) *Vascular dilatation with beading* occurs: (1) at the site of thrombi in arteries and veins and in areas of local immobilization of blood; (a) in primary arterial, arteriolar and venous occlusion; (b) in brain abscesses; (c) in carbon monoxide poisoning (acute stage); (d) in kerosene poisoning; (e) in "rat paste" (phosphorus and sodium fluoride) poisoning; and (f) in electrical trauma: (2) in the localized varicose deformities of the vascular bed typical of Wernicke's superior hemorrhagic polioencephalitis in chronic alcoholism: (3) in the sinusoids of brain tumors. (C) *Hemorrhages* are found: (1) in arterial and arteriolar disease; (2) following venous occlusion; (3) following mechanical trauma; (4) in poisoning (arsphenamine, carbon monoxide, nitrous oxide, kerosene, rat paste, alcoholism); (5) in acute hemorrhagic necrotizing leukoencephalitis and other postinfectious encephalitides; (6) in syphilitic gummatous meningoencephalitis. (D) *Capillary anemia, with or without hyperemia of larger vessels, sometimes mixed with hyperemic zones either in the center or at the periphery of the lesions,* to be found: (1) in softening and demyelination from arterial and arteriolar occlusion; (2) in areas of early demyelination from venous occlusion, e.g. in thrombosis of meningeal veins in purulent meningitis; (3) in multiple sclerosis; and (4) in experimental local anaphylaxis. (E) *Destruction of vessels of first and second order (capillaries and immediate precapillaries)* namely *partial capillary and precapillary avascularity,* is found: (1) in cerebral scars (a) following arterial and arteriolar occlusion, (b) following traumatic laceration, and (c) following carbon monoxide poison-